MONOGRAPHS ON EXPERIMENTAL BIOLOGY

EDITED BY
JACQUES LOEB, Rockefeller Institute
T. H. MORGAN, Columbia University
W. J. V. OSTERHOUT, Harvard University

CHEMICAL DYNAMICS OF LIFE PHÆNOMENA

BY

OTTO MEYERHOF

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BY

PROF. OTTO MEYERHOF

KIEL

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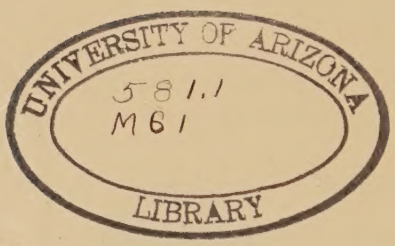

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EDITORS' ANNOUNCEMENT

The rapidly increasing specialization makes it impossible for one author to cover satisfactorily the whole field of modern Biology. This situation, which exists in all the sciences, has induced English authors to issue series of monographs in Biochemistry, Physiology, and Physics. A number of American biologists have decided to provide the same opportunity for the study of Experimental Biology.

Biology, which not long ago was purely descriptive and speculative, has begun to adopt the methods of the exact sciences, recognizing that for permanent progress not only experiments are required but that the experiments should be of a quantitative character. It will be the purpose of this series of monographs to emphasize and further as much as possible this development of Biology.

Experimental Biology and General Physiology are one and the same science, by method as well as by contents, since both aim at explaining life from the physico-chemical constitution of living matter. The series of monographs on Experimental Biology will therefore include the field of traditional General Physiology.

Jacques Loeb,
T. H. Morgan,
W. J. V. Osterhout.

PREFACE

Some of the five lectures of this volume were delivered in Cambridge, England, in the summer of 1922, before the research workers of the physiological laboratories. With the exception of the first * they were also presented in a more complete form in the spring of 1923, at the Rockefeller Institute in New York, as well as before the physiologists of several American universities. The second one formed the subject of a "Harvey Lecture" before the New York Academy of Medicine.

The title for the volume of lectures was chosen to express the deep gratitude which I owe to the late Dr. Jacques Loeb, who by his book on *The Dynamics of Living Matter* gave the decisive stimulus for a new awakening of cellular physiology, from which resulted the most fertile connections of our science with physical chemistry.

The lectures are not intended as an exhaustive treatment of the subject, since they contain only part of the results which have been brought to light on the metabolism and the energetics of cells by German and English investigators working along these lines, and since even the sum total of these results hardly represents a complete chapter. Nevertheless I yielded to the suggestion of Dr. Loeb that a publication of the lectures might be welcome to American physiologists and biologists. On account of the special occasions for which these lectures were prepared, each of them had to be more or less independent of the rest. In spite of this the reader will not fail to notice that they are connected with each other.

* The first lecture was printed in the original form in *The Lancet* (1922). I am indebted to Prof. F. G. Hopkins, Cambridge (England), for revising the text of this lecture.

The lectures include besides my own studies also part of the work of Otto Warburg, Berlin-Dahlem, to whom I owe my personal introduction into the problems of cellular physiology. The dynamics of muscle are treated in close connection with the results of the Cambridge School, particularly those of A. V. Hill.

To avoid repetition, some parts of the original lectures have been omitted, while some additions have been made concerning more details and the most recent results in this field of work.

I wish to express my great obligation to Dr. J. H. Northrop and Dr. H. E. Himwich for the revision of the English text.

April, 1924. O. M.

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CHEMICAL DYNAMICS OF LIFE PHÆNOMENA

CHAPTER I

ON THE PHYSICOCHEMICAL MECHANISM OF CELL RESPIRATION

In this lecture it is intended to discuss some general principles of cell respiration, and it seems therefore desirable to state first which problems appear most vital in this connection. Respiration is no mere external attribute of living matter; it is a central life process, supporting the entire complicated machinery of the living being.

If we desire to reduce the processes of life to physical and chemical terms, we must inquire as to what it is that distinguishes respiration from the chemical reactions of the inorganic world. There seem to be two important differences. The substances broken down and mainly oxidized in the animal body are not in true equilibrium with the oxygen of the air, when they are outside the body; they are in a false equilibrium, for on the removal of impediments to reaction—for example, under sufficient rise of temperature—with oxygen, they yield carbon dioxide, water, sulphuric acid, ammonia, etc.

Such impediments prevent protein or sugar in aqueous solution from being burnt by the oxygen of the air outside the organism. What are the circumstances which in the case of the cell remove resistance to reactions and increase their velocity to the height of oxygen respiration? The general answer that it is done by cell enzymes or catalysts cannot satisfy us. First, we want to see their

existence demonstrated; and second, we have to do with special chemical processes which are distinguished also in other respects from the processes of inorganic nature. The respiration yields energy for doing work, and these work-producing chemical processes not only possess a large store of available free energy, but also put it more or less clearly at the disposal of the cell. We know that in this case energy is changed into work, not by way of heat, but directly; this fact postulates a definite order in, and organization of, the chemical process. This direct transformation requires a machine-like arrangement or structure—as seen, for example, in a galvanic element, where chemical energy does not pass through heat but directly into electrical energy. Of course, the transformation of heat into mechanical work in the case of the steam-engine also requires a machine-like arrangement, but there the chemical processes included in the heating of the boiler need no elaborate organization.

The two problems now presented—the cause of the reaction velocity of vital oxidations, and the transformation of the oxidation energy into work—are closely connected with the structural properties of the cell. The connection is obvious as regards the transformation of energy, but there also exists a close connection between speed of respiration and cell-structure. This fact has been brought out by the investigations of Otto Warburg.[139] In these investigations, which I am now going to discuss, I myself have taken an active part in the Heidelberg laboratory and elsewhere.

That there must exist a connection between the solid stainable structures of the cells, and the velocity of oxidation, and that respiration is essentially dependent on this structure, can be demonstrated in various ways. I may quote, as an example, the fact that the nucleated red blood corpuscles of birds show a much larger consumption

of oxygen than the non-nucleated corpuscles of mammals.[133] Furthermore, the younger blood corpuscles of birds, the protoplasm of which possesses reticular structure which can be deeply stained with methylene blue, consume more oxygen than the older ones. The result of the following experiment is still more convincing.[136] If the nucleated blood corpuscles of birds are hæmolyzed, being first frozen and then thawed out again, respiration does not decrease at all. Through the microscope it can be seen that the nuclei are intact, while the cell-membrane is perforated and the hæmoglobin and fluid protoplasm are mixed; if this cytolyzed cell suspension is now strongly centrifuged, two layers will be obtained—the upper layer containing the hæmoglobin and the fluid protoplasm, the lower one the solid cell particles, chiefly the nuclei. Respiration takes place entirely in this lower layer.[137] But if the nuclei are disintegrated in a crushing apparatus, respiration ceases completely. Over ten years ago Warburg carried out these experiments in England by means of the cell-crushing apparatus designed by Barnard and Hewlett.

Another advance in the analysis of the relations of structure and respiration was made by experiments with sea-urchin eggs, carried out largely by Warburg and myself. If the egg is fertilized or subjected to treatment which produces an artificial parthenogenesis, *e.g.*, short exposure to valerianic acid in sea-water,[66] the consumption of oxygen rapidly increases, as much as eight- or tenfold, with the formation of the fertilization membrane and the organization of the differentiated cell substance.[134] Quite recently Mr. Shearer, of the Cambridge Physiological Laboratory, found an eightyfold increase during the first minutes after fertilization, and made at the same time the interesting discovery that this rise set in on the mere contact of the sperm with the surface.[122]

The production of carbon dioxide and heat also rises in proportion.[75, 123] Thus, the intake of oxygen in this instance expresses typical respiration. If the sea-urchin's eggs are destroyed by mechanical means, the result is apparently very different from that obtained by erythrocytes. The eggs can very easily be reduced to a shapeless pulp in which not even the outline of an egg is to be seen. This can be done simply by rubbing them with sand [108] or by adding distilled water,[79] or still more thoroughly after removal of their albuminous membrane by strong shaking of the coagulated egg-suspension.[141] If these experiments are tried on non-fertilized eggs, the oxygen consumption does not decrease at all; it even increases a little. If, on the other hand, fertilized eggs are cytolyzed, the increase of respiration, produced by the fertilization, falls off again and the juice of the fertilized eggs now respires exactly at the same rate as that of the same quantity of unfertilized eggs. This result, however, does not contradict the result obtained with the blood corpuscles of birds, as it may appear at first glance to do. The cell-pulp of the sea-urchin's eggs no longer shows structures of a coarse kind, but consists for the most part of fine granules. Here, too, it can be shown by centrifuging that the respiration is essentially dependent on these granules.[141] But the cortical layer of the eggs newly formed through fertilization, and all the coarser structures, disappear on cytolysis. Consequently the increase of the oxygen intake after fertilization is abolished. Warburg was able to show, in various ways, that the cortical layer of the eggs is indeed of special importance in the acceleration of oxidation; for instance, substances which can be proved not to penetrate into the eggs, but to work only from the outside—*e.g.*, caustic

soda in sea-water—can considerably accelerate the respiration of the egg.*

Leaving for the present further investigation of the respiration in the cytolyzed eggs of sea-urchins, I will now quote another series of experiments to show how the apparently mysterious influence of structure upon the velocity of oxidation may be interpreted. If respiring cells are put into a solution of an indifferent narcotic, their oxygen consumption will be stopped in a reversible manner. For the effective narcotic concentrations we find here the same law as Overton and Hans Meyer had previously found for anæsthesia of the brain of tadpoles—that is to say, the higher members of homologous series are effective in much smaller concentration.[73, 116] Their effective power is, therefore, increasingly larger than that of the lower members; this is indicated in Table I on the homologous series of alcohols and

TABLE I. RULE OF HOMOLOGOUS SERIES.

Substance.	Partition coefficient oil—water. (Overton.)	Stopping of oxidation. Molecular concentration. (Warburg.)	Critical molecular concentration for narcosis. (Overton.)
Methyl alcohol	2:∞	5.	0.62
Ethyl alcohol	1:30	1.6	0.31
Propyl alcohol	1:8	0.8	0.11
Iso-butyl alcohol	∞:12	0.15	0.045
Iso-amyl alcohol	∞:2	0.045	0.023
Heptyl alcohol	∞:0.15	0.01	.....
Octyl alcohol	∞:0.05	.....	0.0004
Acetone	slight: ∞	0.9	0.26
Methyl-ethyl ketone	∞:20	.....	0.09
Diethyl ketone	∞:5	.....	0.029
Methyl-propyl ketone	∞:4	0.17	0.019
Methyl-phenyl ketone	∞:0.3	0.014	0.001

* If eggs stained with neutral red are put in sea-water which has been made alkaline by addition of caustic soda, they remain red. They change to yellow, however, if sodium hydroxide is substituted by ammonia. In the former case, therefore, the interior of the egg remains acid although the OH' concentration of the sea-water is higher than in the case of ammonia, nevertheless respiration is increased.[134,136]

ketones for Overton's narcosis as well as for the inhibition of respiration according to Warburg.[135, 139]

Overton and Hans Meyer attempted to explain this phenomenon by the rate of lipoid solubility of these substances, or, more exactly, by the partition coefficient between the cell lipoid phase and aqueous solution. The higher members are more soluble in the lipoid phase and reach, therefore, the same concentration in it at a smaller external concentration than the lower members. Warburg and his co-workers, however, proved by a series of convincing experiments that the increase in concentration in the lipoids cannot furnish the explanation for the narcosis of cell respiration—for in this connection only that single aspect of narcosis is involved. If we determine directly the partition coefficient for the distribution of a narcotic between the living cell and the surrounding solution, under the same external concentrations, much more of the highly effective narcotics than of the less effective ones will be found in the cell.[153] In the erythrocytes, for example, seven times as much thymol will be found in the cell as in the same volume of surrounding solution; on the other hand, only 0.8 times as much acetone will be found inside the cell as outside. If we now hæmolyze the bird erythrocytes, remove the stromata by centrifugation, and determine separately the combination of thymol with the liquid and solid cell particles, the solid particles are found to combine with ten times as much thymol as the liquid particles.[152, 155] Finally the combination of thymol and other higher narcotics with the solid parts is not noticeably decreased if the stromata are completely freed from lipoids by boiling for a long time in alcohol and ether.[152] We can, therefore, completely eliminate the lipoid theory, leaving as an alternative only adsorption.

In the first place, the condensation of narcotics in the solid structural elements of the cells runs exactly parallel with their effectiveness. The adsorption of narcotics on these solid structures explains quantitatively their effect upon the respiration. I. Traube was the first to draw attention to the fact that the narcotic activity of different alcohols varies with their power to lower the surface tension of water. In this lowering of the surface tension Traube saw the cause of their narcotic effect.[129] That there can, however, in this case be no direct, but only an indirect connection, is seen from the fact that the urethanes, or the substituted ureas, lower the surface tension of water considerably less than to the degree which corresponds to their narcotic activity. It is the actual power of adsorption on boundary surfaces which counts. Warburg first recognized this in the precipitation of protein which occurs in the press juice of yeast on the addition of narcotics. The degree of precipitation runs exactly parallel with the rate of inhibition of fermentation. (I would add that alcoholic fermentation is influenced by narcotics in the same way as oxygen respiration.)[151] Imagine, now, the fermentation enzyme in Buchner's yeast juice, the zymase, as a highly colloidal protein, and let the fermentation reaction proceed on the surface of this colloid. The inhibitory effect of narcotics can be clearly explained, according to Warburg, by the diminution of the effective surface of the enzyme, which accompanies the precipitation. As I have shown in publications on the narcotic inhibition of ferment processes, we are dealing with an exaggerated expression of the effect of narcotics. The precipitations are caused by salt, but the proteins are sensitized to aggregation by the presence of the narcotic.[92] In the absence of salt these precipitations do not occur, though a narcotic

inhibition of enzymes in the yeast extract can still be shown.[91] Indeed, in a solution of invertase free from protein [79] and, finally, in the case of an inorganic enzyme model, colloidal platinum,[80] I demonstrated similar narcotic inhibitions and the validity of the rule of the homologous series, though no diminution of the number of particles could be shown in the ultramicroscope. In Fig. 1 are shown the inhibiting effects of the invertase action

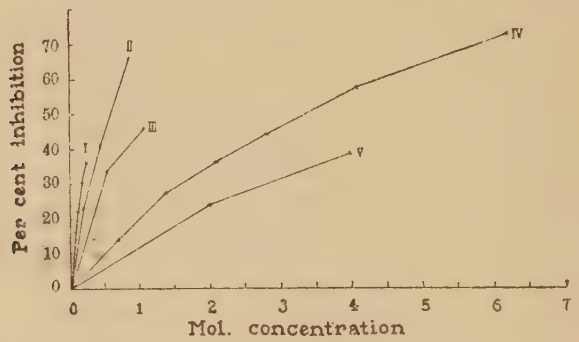


FIG. 1.—*Narcotic inhibition of invertase by alcohols.* I, amyl alcohol; II, *i*-butyl alcohol; III, *n*,-propyl alcohol; IV, ethyl alcohol; V, methyl alcohol.

by alcohols. The curves show the typical shape of the adsorption curves, slightly concave towards the abscissa axis. For these inhibiting effects I suggested as an interpretation, that the more strongly adsorbed narcotics drive off the substrate from the colloidal enzyme surface, but that the dispersion rate of the enzyme does not thereby undergo any change. This is obviously the case in any reversible narcosis.[163, 57a]

Cyanide also inhibits respiration in a reversible manner even in a molecular concentration of 1/10,000. It is, therefore, considerably more effective than the strongest indifferent narcotics; but it is only slightly adsorbed, and its high activity must be explained in another way.[138, 140]

The next assumption is a chemical combination of cyanide with some substance, present in a minute quantity, but yet essential for respiration, hence most likely with an oxidation catalyst of the cell. During the experiments with the cell-pulp of sea-urchin eggs our attention was drawn to the iron, which, as is well known, can easily enter into a complex compound with cyanide. The fact could be established that the oxidation of lecithin in presence of ferrous salt is the chief or initial process of respiration in the eggs.[154] The chemical aspect of this process will be discussed in the next lecture. I will touch here only upon the following points concerning the bearing of iron on respiration.[140] First, the sea-urchin eggs contain per 1 gm. of cell-substance, about 1/500,000 mol. of iron in such a form that it yields a red color with hydrochloric acid and potassium thiocyanide. It therefore exists already in ionic form or is very easily split off as such. Second, an addition of iron salt, in quantities corresponding to those existing in the egg, increases the respiration considerably. Very much larger quantities do not produce any stronger effects. On the other hand, additions of the metal much smaller than the quantity present in the egg are ineffective. Third, the increase of oxidation caused by the addition of iron is inhibited in the same way as the normal respiration, by narcotics.

How, then, shall we interpret the influence of structure on the one hand, and iron on the other, as coöperating in the mechanism of respiration? One hypothesis which offers itself is, that respiration occurs on structural surfaces of macroheterogeneous and microheterogeneous systems, containing iron. The hypothesis, that the high velocity of oxidation of the foodstuffs in the cell is based

upon a catalysis on surfaces containing iron, has been very recently confirmed by Warburg in a striking manner on a respiration model, Merck's blood charcoal suspended in aqueous solution.[142, 145, 157]

It is well known that amino-acids in aqueous solution are perfectly stable when exposed to the air. If, however, animal charcoal is added to an amino-acid, the amino-acid is burnt at the temperature of the body in presence

TABLE II. CONCENTRATION OF NARCOTICS NECESSARY TO REDUCE THE OXIDATION OF CYSTINE BY CHARCOAL TO 40 PER CENT. OF ITS INITIAL VALUE.

Substance.	Concentration in solution mols. per liter.	Adsorbed millimols per gm. charcoal (x).	Covered area $x.V\frac{\text{Å}}{m}$.
Dimethyl urea (asymm.)...	0.03	1.1	9.0
Diethyl urea (symm.).....	0.002	0.68	6.9
Phenyl urea.....	0.0002	0.76	8.2
Acetamide.....	0.17	1.2	7.3
Valeramide.....	0.003	0.62	6.9
Acetone.....	0.073	1.33	8.3
Methyl-phenyl ketone.....	0.0004	0.73	8.0
Ethyl alcohol.....	0.32	....	...
Amyl alcohol.....	0.0015	0.87	7.9
Acetonitrile.....	0.2	1.5	7.7
Valeronitrile.....	0.0021	....	...

of the oxygen of the air, without any other addition. It is burnt to the same end-products as in the body, *e.g.*, cystine into carbon dioxide, sulphuric acid, and ammonia. Nitric acid is never formed as in the combustion in the Berthelot bomb. Thus, in chemical respects we have obviously before us a process of oxidation very similar to that in the cell. It takes place on the surface of the charcoal, and is inhibited by different narcotics in exactly the same way as the respiration in living cells.

In Table II are indicated the first and last members of five homologous series, and in the second column the molar concentrations, which inhibit the oxidation rate of

cystine by exactly 60 per cent. Here we see the marked decrease of the effective concentrations as we ascend the homologous series, which indicates an enormous increase of the active power in the same direction. Now Warburg was able to prove that the inhibition is caused by driving off the cystine from the surface of the charcoal. When the inhibition amounts to 60 per cent. (Table II). we also find exactly 60 per cent. of the absorbed cystine driven off from the charcoal, as is shown by adsorption measurements with narcotics and without. If we now determine the amounts of the various narcotics which are adsorbed by the charcoal, when in each case the oxidation velocity is reduced by a constant amount, we find the values given in the third column. It will be noticed that the quantity adsorbed very nearly agrees in millimols per gm. charcoal, while the surrounding concentrations, differ about a thousandfold. Nevertheless, this agreement is not perfect, as the first members of the homologous series are always adsorbed a little more than the last members before they induce an equal degree of inhibition. But this last trifling deviation disappears completely when we consider that the rate of removal of cystine depends obviously upon the area covered by the narcotic on the charcoal surface. Warburg adopts the suggestion of Langmuir, that the adsorbed substance covers the surface in a monomolecular layer.[62] Starting from this supposition, he calculates the space occupied by each single narcotic on the surface. Since the larger molecules of the higher members need a larger space than the lower ones, the numbers of Column three must be multiplied by a factor for the surface covered by each single molecule, and this factor increases with the higher members. If we imagine the molecules of spherical shape and

determine directly the molecular volume of each substance, by measuring the molecular refraction,* we get the numbers shown in Column four for the surface covered by each narcotic at a corresponding rate of inhibition. It will be seen that the number is constant within the limits of experimental accuracy (V_m = molecular volume, x the number of millimols, so the covered surface is equal to $x.V_m^{\frac{1}{3}}$).

The charcoal model imitates the inhibition by cyanides as perfectly as the inhibition by narcotics. The oxidation of the cystine is inhibited by n/10,000 to n/100,000 cyanide, though cyanide is very slightly adsorbed. Although, therefore, only a minimum part of the cystine is driven off from the charcoal, its oxidation is, nevertheless, completely stopped. Warburg suggests that the cyanide covers, not the whole surface, but exclusively those parts of the surface containing iron, and that the oxidation of the amino-acid only takes place on these. Indeed, the animal charcoal contains iron, about 5/1,000,000 mol. per grm. and the cystine oxidation is stopped by 10/1,000,000 mol. of cyanide. Therefore both values correspond exactly. As a fact, the mechanism of cyanide effect and the way in which iron is active in charcoal, was recently explained still more clearly.**

In the first place, Warburg demonstrated that charcoal made from pure benzoic acid, although a good adsorber of cystine, burns it relatively slowly and the addition of iron salt does not help. If, however, the charcoal was saturated with iron salt and then brought to red heat, the oxidation velocity of this charcoal reached

* According to the theory of Lorenz-Lorentz the molecular refraction $\frac{n^2-1}{n^2+2} \times \frac{\text{mol. weight}}{\text{spec. weight}}$ is equal to V_m, n meaning the refractive index of the substance.

** See also the next lecture.

nearly the same value as that of animal charcoal. The heating made the iron an actual component of the charcoal surface.

Comparative experiments with blood charcoal and pure sugar charcoal brought out further results.[147] If blood charcoal is heated with hydrochloric acid in a sealed tube, thereby freeing its surface from iron, the amino-acids certainly burn on its surface, but the process is only weakly inhibited by cyanide. Now pure sugar charcoal shows no specific inhibition by cyanide at all, although amino-acids and oxalic acid burn on it too. This can be explained in the following way: The charcoal washed free from salt shows even in a neutral solution strong autoxidation, while—as I demonstrated in coöperation with Weber,[109] blood charcoal does so only in an alkaline solution, *i.e.,* in proportion to the adsorbed OH'-quantity. The salts of the blood charcoal, therefore, inhibit the carbon from effecting oxidation in a neutral solution. By bringing pure sugar charcoal to red heat with appropriate salts, Warburg could indeed completely stop its capacity to burn amino-acids and also protect it against autoxidation. While, therefore, in the charcoal, free from salt, carbon itself burns the amino-acids, but is not inhibited by cyanide, the blood charcoal, containing salt, requires the presence of iron in suitable form which acts as oxidation catalyst. It is very peculiar, however, that it also depends on the chemical combination in which iron is contained therein. While the heating of sugar charcoal with common salts of iron is only slightly effective, sugar charcoal brought to red heat with hæmatine gives a material more active than blood charcoal itself.[158a] This might point the way towards the elucidation of enzyme activity.

The application to living cells we might summarize

thus: We may imagine the structural surfaces of the cell to be like the charcoal surface, a mosaic of fields with and without iron, iron in definite chemical combination, similar to hæmatine (pyrrol nuclei?). It may, therefore, be considered that an essential cause of the high velocity of reaction of the foodstuffs in the cell is their adsorption on the surfaces of structures containing iron. We are here dealing with a special instance of surface catalysis. Whether this is the only reason of the high velocity of reaction is still undecided. In this connection it is a peculiar fact that for oxidation on charcoal, the amino-acids occupy an especially prominent place among the stable substances. I have recently investigated the behavior of carbohydrates on charcoal.[109] Curiously enough, glucose is perfectly stable. On the other hand, hexose-phosphoric acid is oxidized in a noticeable amount, although much less than the amino-acids. Fructose is oxidized still less, and lactic acid almost imperceptibly. In whatever way this varying behavior of the carbohydrates be explained, it is interesting to note that in this connection hexose-phosphoric acid proves more easily oxidizable than glucose.

CHAPTER II

AUTOXIDATIONS IN THE CELL

Only about a generation ago physiologists could still believe that through the study of the different functions in higher animals the veil of the processes of life would be lifted more and more. But as fascinating and elucidating as the investigations proved in many directions, and as much stimulus as the clinic derived from them, the principal question of the biologists did not get any nearer its answer in this way. For this principal question probably has been and will be for all times: What is the real character of the processes of life, or by what physical and chemical means can animate nature create and preserve life? I will not touch here upon the philosophical problem, even if this question could be answered unreservedly, nor enter into a discussion with those who declare it to be formulated wrongly, as, for example, do the vitalists. As experimental physiologists we believe that we can approach the answer to our question, but in a way somewhat different from the typical manner of the older school of physiology. We study the events of life under the simplest conditions possible, singling out what is common to all of them and trying to control them by every means that physics and chemistry offer.

The more closely we study the machinery of life in the isolated cell, the clearer it becomes that the chemical and physicochemical processes predominate in it. Physiology in its classical period was studied chiefly by physicists, at least in Germany. I mention Helmholtz, Dubois-Reymond, Ludwig, Fick. In the field of cellular physiology, however, physiological and physical chem-

istry have to assume the leadership—not, however, by analyzing the dead material in regard to its constituents, but by studying the chemical dynamics of the processes of life. Almost a hundred years ago, Pasteur struck out on this path. With extraordinary success, Jacques Loeb, whom many of our younger generation call their teacher, has continued along these lines.

In the first lecture I discussed the problem of cell respiration as one of the most essential life phenomena; indeed, it has continuously occupied the scientists in this field of work. I need not discuss here the numerous solutions propounded within the last century. They often rest a good deal upon speculation and not upon experiments. Again and again the question was put, whether the instability of the organic molecules towards oxygen in the animal was due to a primary change of these molecules or to this oxygen itself. Within the last years, speculation has suggested that not the oxygen, but the hydrogen removed from the organic molecules, was activated.[161] In regard to this we must remember that the physicists cannot yet fully explain the cause of the simplest reaction, not even, for example, how the molecule of hydrogen chloride is formed from one atom of hydrogen and one of chlorine. We should therefore be able to consider our problem of cell respiration as solved if we succeed in basing it on the chemical events of inanimate nature and leave the rest to the chemists.

With one of the steps in this direction we have dealt before. Now I intend to turn to another class of reactions, different from those treated in the last chapter. There is no doubt that an important part in the respiratory mechanism is assignable to autoxidations in the cell which are probably somehow connected with the adsorption catalysis discussed above. As is known, Professor Dakin has described a series of most important autoxida-

tions of this kind.[8, 9, 10, 11, 12] He succeeded in oxidizing amino- an fatty acids in a way similar to that in the animal body by the use of hydrogen peroxide and iron salt.* These reactions doubtless furnish fundamental aspects for the problem before us. I will, however, restrict myself here to two other cases of autoxidizable systems. They are distinguished by the fact that the components are actually in the cell, and that here, as in respiration, only the molecular oxygen of the atmosphere is used for the oxidation. By an "autoxidizable system" I mean one, the components of which by themselves are almost stable in the air; but on their combination rapidly absorb oxygen. For the cases I am going to describe, it is especially interesting that one of the two substances may be present in a comparatively minute amount, because it is able to transfer the oxygen to the other body, and thereby reduce itself. In this case, therefore, the substance present in small quantity works as catalyst.

Such a system was found in the sea-urchin egg by Warburg and myself ten years ago in a common investigation, as partly described before.[108, 154] Warburg continued studying it.[140] If the unfertilized eggs are destroyed mechanically by grinding them with sand or thoroughly shaking them, their oxygen respiration does not stop, but even increases a little. In the beginning also a little carbon dioxide is formed, which process, however, soon ceases, while the consumption of oxygen lasts for some time. The same phenomenon can be observed by precipitating the eggs with acetone and drying them with ether. The acetone powder of the eggs also consumes oxygen with about the same speed as the living cells. Respiration and life of the cell can therefore be completely separated here. In the analysis of this process it

* According to an observation by Warburg, the iron salt can be dispensed with in the oxidation of amino-acids. With H_2O_2 alone the same end-products are obtained.[147]

was found that its occurrence depends on lecithin, or more generally on phosphatides of the egg. It depends besides on a small quantity of iron, contained in the egg as salt or in a very loose combination, and finally on a slightly acid reaction of the egg substance.

The autoxidation of lecithin under the influence of iron salt was first studied by the Swedish physiologist Thunberg.[125, 126] The spontaneous consumption of oxygen of his lecithin preparations was about trebled by the addition of 1/1000 mol. of iron salt. In the purer lecithin preparations, however, the spontaneous consumption of oxygen is considerably less than in his experiments. Indeed, even in the course of several hours it could hardly be measured. In this case the oxidation velocity is increased several hundred times by a millimol of bi- or trivalent iron. Warburg has studied this autoxidation more closely, both in a chemical direction and in regard to its occurrence in the egg of the sea-urchin. I will quote some of his results. They will also be of value to us in the new autoxidation process to be described later.

As regards the chemical reaction itself, it was found that of all the components of lecithin and numerous unsaturated fatty acids, the linolenic acid alone with its three double linkages shows the same autoxidation with iron. During this, the number of double linkings decreases, a reaction is going on which is given in the equation below.

Linolenic acid: $C_{18}\ H_{30}\ O_2$

$CH_3.\ CH_2.\ CH{=}CH.\ CH_2.\ CH{=}CH.\ CH_2\ CH{=}CH.\ (CH_2)_7.\ COOH$

$$\begin{array}{l} CH \\ \| \\ CH \\ | \end{array} + O_2 + H_2O = \begin{array}{l} CHOH \\ | \\ CHOH \\ | \end{array} + O = \begin{array}{l} CHO \\ | \\ CHO \\ | \end{array} + H_2O$$

About double the quantity of oxygen is added to the acid molecule as is necessary for the formation of dihydroxy acid. Evidently peroxides are formed, as is known from the spontaneous autoxidation of these unsaturated fatty acids. CO_2 is not produced, and, anyhow, no further decomposition of the oxy-acids seems to occur.*

That this process actually starts the respiration of the sea-urchin egg, Warburg could demonstrate as follows: In each gram of dry substance of the egg, there are present some 1/100 mg. of iron, as was discussed in the former lecture. If now the same amount of salt is added, the respiration is doubled. The respiration increased by iron is similar to the original one. Thus, for example, it is stopped by the same concentrations of narcotics as the respiration. On the other hand, the participation of linolenic acid in this process is also obvious. If linolenic acid is added to the fresh suspension of the destroyed eggs, it has no effect, the respiration does not change. If, however, linolenic acid is added three to four hours later, *i.e.*, at a time when the spontaneous respiration is markedly decreasing, the respiration rises again to its original height. Evidently the linolenic acid, present in the egg before, has now been consumed. The addition of new substrate for the oxidation raises it again.

Certainly, the autoxidizable system, consisting of linolenic acid and iron does not exhaustively explain the respiration of the unfertilized egg. It merely shows the point of first attack of oxygen. For in the test-tube, the

* S. Coffey[7] recently demonstrated that on spontaneous autoxidation of linolenic acid, spread over glass plates in a thin layer, 9 atoms of oxygen were taken up per molecule (at 100°). Hereby 1 mol. of CO_2 and 1 mol. of acetic acid are formed, while the following decomposition is taking place:

$$C_2H_5 . \underset{|}{CH} — \underset{|}{CH} . CH_2 . COOH + CH_3 . COOH + CO_2 + CHO[CH_2]_7 . COOH .$$
$$O —— O$$

At the same time a positive reaction on peroxide is obtained.

reaction stops on the conversion of linolenic acid into slightly oxidized products. In the egg substance, however, it progresses as far as the formation of carbon dioxide. Moreover, the respiration of the unfertilized egg can be inhibited by narcotics in a typical manner. We may consider it, as does Warburg, a proof that adsorption catalysis participates in the respiration. On the other hand we can consider the system linolenic acid-iron ion in vitro as homogeneous, and therefore not influenced by narcotics.

After having seen that an autoxidizable system can at least in one case be made responsible for the introduction of cell respiration, I now turn to another such system. Here we must begin with an entirely different starting point, although we shall return afterwards in a startling manner to the system just described.

In 1921 Professor Hopkins, of the great Cambridge School, spoke in his "Herter Lecture" in Baltimore, on his memorable discovery of an autoxidizable constituent of the cell, hitherto unknown.[58] He called it "glutathione" and made out that it was a dipeptide consisting of cysteine and glutamic acid. The following is the formula proposed by him:

$$HS.CH_2.CH.NH_2.CO-NH.CH.CH_2.CH_2.COOH$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad COOH.$$

On this occasion he gave a most impressive synopsis of the general theories of autoxidation and especially of the earlier history of his discovery. I need not repeat, therefore, his statements, especially as in the meantime a series of further publications by Hopkins and his pupils has appeared.[14, 59, 60] I may merely restrict myself to a few remarks. The idea of ascribing to the sulphydryl group of the cysteine a share in the respira-

tion, originated with the Berlin pharmacologist Heffter.[40] A number of investigators, Arnold,[1, 2] Thunberg,[127, 128] and in America Mathews and Walker,[70] took up this idea and went on working with the reaction of the SH group. Owing to the interruption of international scientific intercourse until shortly after the war, Hopkins had at first remained in ignorance of my own work in this field. Since then, however, he has given an appreciative report of it, comparing it with his own results. Indeed, my experiments with this reaction on similar objects to his had resulted in a coincidence in many points. In a brief summary I shall present the results of my experiments of that period before dealing with the later ones.[86, 87]

Just as in the acetone preparations of the sea-urchin's eggs, so also in the acetone yeast, made according to the technique of the German chemist Buchner,[5] a constant consumption of oxygen can be demonstrated. By numerous analogies it proves to be a partial process of the yeast respiration. The same also holds good for the yeast press juice perfectly free of cells, and for the so-called maceration juice of the yeast, made according to a technique of the Russian chemist Lebedew.[64a] Now, by washing with water, the respiration of the yeast can be abolished, by the addition of this aqueous extract it is revived. In a similar manner the oxidation in the maceration juice can be abolished by ultrafiltration and be reëxcited by the addition of the ultrafiltrate. In place of this ultrafiltrate, the filtrate from the boiled extract, the so-called "boiled juice," may be used. This observation, which I could extend to animal tissue, muscle, and liver, will be treated more in detail in the next chapter. I here emphasize only an accompanying observation which was made during these experiments with great regularity. One compound, causing the sulphydryl reaction, always passes into the

aqueous extract upon the washing or ultrafiltration, while the residue becomes free of SH. This could easily be seen in the very sensitive Arnold reaction, the purple coloring with nitroprusside and ammonia.[1] This SH body, as we now learn from Hopkins, is nothing else than glutathione. Now it was striking that the power of the extracts for reactivation of the residue formed a parallel with its contents of SH groups, especially after different kinds of treatment of the extract, as heating, incubation with alkali, precipitation with alcohol, etc. This idea was developed further and it was proven that with added SH compounds oxygen could indeed be transferred to washed acetone yeast. I used chiefly thioglycollic acid

$$\begin{array}{l} CH_2SH, \\ | \\ COOH \end{array}$$

which is more stable in neutral reaction than cysteine. In the mixture of acetone yeast plus thioglycollic acid there was consumed several times as much oxygen as would have been required for the transition of SH into disulphide, according to the formula $2RSH + O = RS - SR + H_2O$. It was especially curious that the O_2 transfer succeeded best in a weakly acid solution where the thioglycollic acid was relatively stable. On the other hand, in a weakly alkaline solution, where it is itself autoxidizable, the O_2 transfer quickly ceased (Fig. 2).

All the same, even at that time, certain definite points were found showing that this transference of oxygen must not be considered the same as the respiration of the killed yeast, but that we have at best only part of the process before us. For the spontaneous oxygen consumption of the yeast preparations is, like all respiration, sensitive to heat. This holds good also in the case where this respiration is separated into two components by extraction with

water. The yeast residue which, as we must suppose, contains the respiratory enzyme, is thermolabile, and only the components, contained in the water extract, which I have termed "respiratory substance," are stable under boiling. On the other hand, it was established that the sulphydryl groups transport the

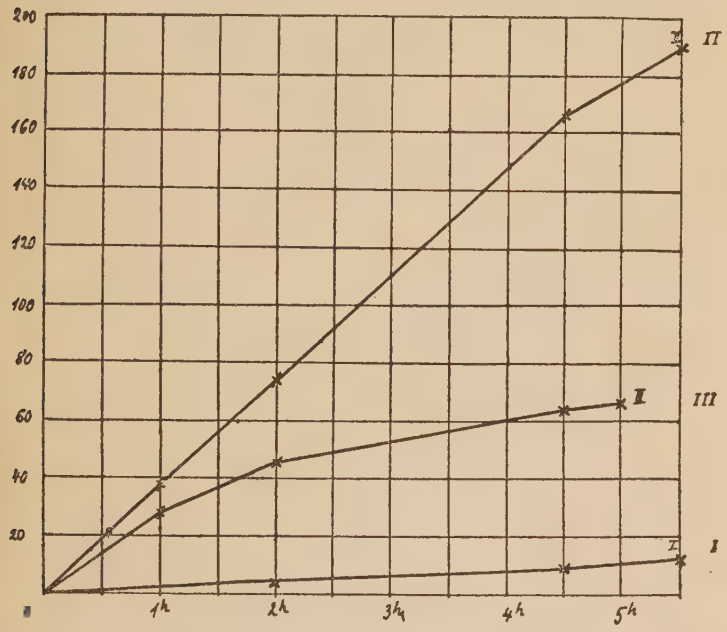


FIG. 2.—*Rate of oxidation of washed acetone yeast in the presence of* SH. Ordinates are the cubic millimeters O_2; abscissae, the time in hours. I, without addition of SH; II, with thioglycollic acid (neutr.); III, with thioglycollic acid (alkal.).

oxygen to a constituent of the yeast residue, which was itself stable upon boiling. Furthermore, this O_2 transport could not be influenced by narcotics and therefore could not depend on the cell structure, unlike respiration. The only conclusion possible was that a purely chemical process is taking place here in which the oxygen is transferred to a definite chemical constituent of the cell.

At this point my investigations of that period stopped, and it was only by Hopkins' discovery that I was stimulated to take them up again. Then, in the meantime, Hopkins himself had also found out that glutathione transports oxygen to a system stable under boiling, and that, apart from delicate differences, no essential distinction existed between the two processes being studied by him and by myself.* He had worked chiefly with preparations of muscle dried with alcohol and ether, *i.e.*, prepared in a similar manner to acetone yeast. As the next lecture will show more in detail, there exists doubtless an especially close affinity between the metabolism in the yeast and muscle tissue.

After having established that the "preformed sulphydryl system," present in the cell is also stable under boiling, it appeared as the most important task to find the components to which the oxygen is transferred, and thus to demonstrate the autoxidizable system in vitro. It was certainly discouraging that Hopkins and his co-workers had not reached any definite results, but two circumstances came to my aid. These were, that the process had been studied by myself in a weakly acid medium and with the addition of the reduced sulphydryl compound, and not of the disulphide formed from it. To be sure, as Hopkins proved, disulphide also is capable of oxygen transfer being in turn reduced by the cell-substance. Indeed, he himself has used as a rule glutathione in the disulphide form. However, this reduction, as I

* In my former publications I had advanced the idea that the disulphide formed from thioglycollic acid could not transport oxygen, while Hopkins' glutathione could do so even in its oxidized state. But these older statements of mine are not exact. The dithiodiglycollic acid transfers oxygen more slowly, but in a measurable amount, and can be reduced in vacuo to the sulphydryl compound. Also on this point there exists therefore no essential difference between the glutathione and the thioglycollic acid.

am going to show later, must evidently precede the transfer of oxygen. Therefore the oxidation velocity with the sulphydryl compound itself is considerably greater than with the disulphide.*

Now I found [106] that under the above-mentioned circumstances the oxygen transfer ceases if the dried muscle is being continuously extracted with alcohol and ether. If, however, the alcohol-ether extract is cautiously condensed at room temperature, it takes up oxygen in the

TABLE I. O_2 TRANSPORT BY SH GROUPS ON "LECITHIN."

Substance.	cmm. O_2 needed for disulphide.	O_2 consumed, cmm.	Multiple.
Alcohol-ether extract of heart	11	108	10.
of muscle	27	175	6.5
" "	11	81	7.5
lecithin ex ovo	17	130	7.5
" "	23	173	7.5
Linolenic acid	25	241	10.
" "	23	195	8.5
Linseed oil	18	178	10.

presence of sulphydryl groups. Ten times as much oxygen can be taken up as the transition to disulphide requires.

Further analysis of this process showed that it depends on lecithin or on phosphatides, respectively, and, moreover, that here also linolenic acid must be considered as the only effective compound. Therefore, linolenic acid is oxidized in presence of SH in a similar manner as in presence of Fe. Also in this case, as can be demonstrated,

* It is especially true with a weakly acid reaction, where the maximum of the O_2 transfer is obtained (pH about 5). In a neutral, or weakly alkaline medium (pH 7–9), the oxygen transfer by sulphydryl is very strongly inhibited. These experiments were made by me with thioglycollic acid and partly repeated with cysteine. The former transports far more oxygen to the dried muscle than does glutathione, according to Hopkins' experiments. Thus the examination of the end-products is here very much facilitated.

part of the double linkings of lecithin or linolenic acid, respectively, vanishes during this reaction. An abundance of facts lends support to the idea that the oxygen transfer to muscle by means of sulphydryl groups of thioglycollic acid and cysteine rests upon nothing else but the oxidation of unsaturated fatty acids, especially of the linolenic acid group. In place of many arguments of probability I will only quote one, which we may consider as a crucial test. We extract muscle at room temperature with alcohol and ether, first a control sample which has been before suspended only in water and shaken in air; then a sample to which thioglycollic acid, or cysteine, or disulphide has been added, and where the consumption of oxygen was determined. We then obtain the following results: In the alcohol-ether extract of the first sample without SH, the double linkings of the unsaturated fatty acids, computed by the iodine number, remain constant. In the second sample, however, shaken in the air with SH, they have disappeared for the greater part. The number of double linkages vanished corresponds exactly in the order of magnitude to the measured combination with oxygen. Only as in the case of iron, a little more oxygen has been taken up than corresponds to the oxidation of the double linkages into hydroxyl groups.

That the phosphatides in muscle are indeed present in a condition susceptible to autoxidation under the influence of external reagents, is seen from the fact that an addition of iron to washed muscle likewise produces O_2 consumption with all the signs of lecithin oxidation.

However, there remains one point not completely cleared up by the tests in vitro. Oxygen can be transferred to muscle substance also by means of the oxidized compound, disulphide, in place of sulphydryl, while disulphide with lecithin or linolenic acid is nearly ineffective.

This might seem a very weighty objection if the reaction in muscle really occurred as simply as most of the authors have imagined, *i.e.*, according to the two equations:

$$(1) \quad 4\,RSH + O_2 = 2\,RS - SR + 2\,H_2O$$
$$(2) \quad 2\,RS - SR + 2\,MH_2 = 4\,RSH + 2\,M$$

(M: muscle substance.)

The first equation represents the spontaneous oxidation of sulphydryl into disulphide, the second the reduction of the disulphide into sulphydryl, during which process two atoms of hydrogen are removed from one molecule of muscle substance, marked M. According to this idea, the oxidation of the tissue material is brought about solely by the disulphide. This oxidation is a removal of hydrogen, a dehydrogenation of the kind which the chemist Wieland recently propounded for most biochemical oxidations.[161] According to this theory it remains, however, incomprehensible, how the sulphydryl compound can transport the oxygen in a larger amount, and above all with greater speed, than the disulphide itself. But the reaction becomes clear from the following scheme:

$$(3) \quad RSH + HSR + O_2 = R.S.\overset{O-O}{\underset{|\,|}{H\,H}}.S.R$$

$$(4) \quad R.S.\overset{O-O}{\underset{|\,|}{H\,H}}.S.R + M = 2\,RSH + MO_2$$

According to the above the sulphydryl compound transfers the oxygen to the muscle substance, and temporarily forms a peroxide which yields its entire oxygen to the muscle substance, thereby being itself regenerated. The accepted formula of the peroxide is of course hypothetical. Every single molecule of SH could form a peroxide just as well. Besides the coöperation of a metal

in the peroxide formation is not unlikely on account of the reasons to be discussed later.

Therefore the formation of the simple disulphide does not occur here at all. All the same, a little of it is produced by spontaneous oxidation. In this way the catalyst would be consumed if the disulphide could not be reduced in its turn by muscle. It is true that it passes according to the above two equations (1) and (2), which, in my opinion, do not illustrate the principal reaction, but only a secondary one. In the test-tube only the reactions (3) and (4) are going on. The former (2) obviously depends on special condition in muscle, perhaps on a peculiar distribution of the phosphatides, and the coöperation of other substances. Until now it could not be reproduced in the test-tube. Finally in presence of lecithin, the sulphydryl compounds themselves are also oxidized in small part as far as sulphuric acid. The process, therefore, occurs less regularly than in muscle. But the correctness of the whole statement is corroborated by the far-reaching analogy of this reaction with the one above described, of oxygen transference by iron to lecithin. Here too, we must assume that from ferrous salt a peroxide is formed. The latter transports its entire oxygen to lecithin under regeneration of the ferrous oxide, so that the process can begin anew. In this case ferric salt is also effective; it is probably first reduced to ferrous salt. Accordingly, the oxygen transference by means of iron would occur exactly as by means of sulphydryl, corresponding to the equations given above (3) and (4). On the other hand, the spontaneous oxidation of bivalent to trivalent iron and the reduction of the latter would correspond to the above equations, (1) and (2). In our case we have therefore to suppose an organic

peroxide (itself probably formed by help of some metal) in place of the iron peroxide.

Until now it has not yet been settled whether Hopkins' glutathione works exactly in the same manner as the thioglycollic acid and cysteine tested by me, as there seem to be certain deviations, chiefly in quantitative respects, as well as numerous coincidences. Even if there is no doubt that unsaturated fatty acids are having their share in the glutathione effect, we must consider the possibility of the coöperation of other substances besides.*

Finally, I will mention here one more observation which points to the supposition that linolenic acid represents the most essential oxygen acceptor of muscle in presence of SH. According to Hopkins, washed muscle powder reduces methylene blue under anaërobic conditions very slowly. Glutathione, as disulphide, alone also reduces methylene blue slowly, but combined they do it with great speed in case the reaction is slightly alkaline.

These experiments can be partly imitated with lecithin. For, while the phosphatide does not reduce the disulphide of thioglycollic acid directly in absence of air, it reduces methylene blue in presence of disulphide, and also here the decolorization of the dye occurs more quickly in alkaline than in acid reaction.

It is now an important question to decide whether our system is in any respect whatever at the basis of the respiration of living muscle. There seems to be no doubt from certain considerations that it represents part of the oxygen affinities of the tissue and therefore has its share in the respiration mechanism. On the other hand, one may exclude the idea that muscle respiration is wholly

* After I had delivered this lecture, my statements of the rôle of the SH group of cysteine and of thioglycollic acid in oxidizing the linolenic acid group in muscle were confirmed in all details by Hopkins, using reduced glutathione (*Lancet*, July, 1923).

due to the process described above. This can easily be tested. As we have learnt before, double linkages of fatty acids are always oxidized in muscle in presence of sulphydryl. But it does not happen in a measurable amount in the respiration of muscle, not even when a multiple of oxygen has been taken up, as by the muscle preparations in presence of added sulphydryl.

This last statement seems to me of interest for another problem, *i.e.*, the well-known cyanide inhibition of the oxidation in cells. The hypothesis that the SH group represents an essential factor of cell respiration has given new stimulus to the conception, first expressed by Heffter, that the cyanide inhibition rested upon an obstruction of the SH group by reason of the formation of thiocyanide:

$$R.SH + HCN + O = RSCN + H_2O$$

This hypothesis has been opposed by Warburg, who asserts that the cyanide inhibition of respiration is due in all cases to a removal of catalytic metal, probably iron, by complex combination.[145, 147] For this reason it seemed interesting to me to study more closely the mechanism of the cyanide inhibition in the oxidation of sulphydryl itself.[107] As a result of these experiments it is obvious that in some SH systems there is a peculiar inhibition by small cyanide concentrations which is as strong as in respiration. This inhibition cannot, however, be due to formation of thiocyanide, but must have another cause.

Some years ago Mathews and Walker[71] found that the autoxidation of a 4 per cent. cysteine solution is checked by n/10,000 KCN, that therefore, one molecule of KCN prevents the autoxidation of 2500 molecules of cysteine. On account of this disproportion the authors themselves have discussed the theory, as to whether in the end the inhibition is caused here too by the elimina-

tion of catalytic iron. They have, however, not adopted it, since the presence of iron could not be proven.* For the present, therefore, this case is not cleared up, but I believe I have got an unmistakable result in the similar case of thioglycollic acid. Its spontaneous autoxidation in an alkaline solution is not very sensitive towards cyanide. In a weakly acid solution, however, in the system lecithin plus thioglycollic acid, the effect of cyanide is very strong. Now, as I found, there is only one metal salt which can produce autoxidation of thioglycollic acid in a weakly acid solution, even in the smallest amount, namely, copper salt. Even less than 1/100 millimol of copper produces rapid autoxidation. In this case, for the decrease of the reaction to half the speed there are always needed about 5-6 times as many KCN molecules as there is copper salt. This makes us think that Cu is unionized here according to the equation $2\ CuSO_4 + 10\ KCN = (Cu_2(CN)_8)\ K_6 + (CN)_2 + 2\ K_2SO_4$. This complex compound is well known from the work of the chemist Treadwell.[130]

The formation of such a complex compound can also be proven directly. If KCN in about fivefold concentration is added to a highly diluted solution of copper sulphate, no brown coloring is obtained with ferrocyanide of potassium, *i.e.*, no copper ferro-cyanide is formed. Copper is therefore here no longer present in ionized form. On the other hand, copper sulphate with thioglycollic acid in equivalent quantities gives a purple coloring which is to be considered as the proof of the

* Warburg[148] found that on thorough cleaning, the autoxidation velocity of cysteine greatly diminishes and that it is inhibited by sodium pyrophosphate as much as by cyanide. Sodium pyrophosphate forms stable complex compounds with metals. Therefore it is almost certain that there are also traces of metal responsible for the oxidation, and that the cyanide inhibition is due to their removal. Indeed, by adding 1/10,000 mg. Fe in 10 c.c., the oxidation velocity of the purest cysteine preparation was increased tenfold.

formation of a complex salt of copper and thioglycollic acid.[6] If KCN in about fivefold concentration is added, the coloring immediately vanishes, or does not appear at all. Cyanide, therefore, prevents the complex salt formation of copper with thioglycollic acid, as it also prevents the precipitation of Cu-salt by hydrogen sulphide. The Cu-S linkage is therefore weaker than the Cu-CN linkage. This fact fully explains the inhibition in the case described here.

This explanation probably holds good also for the system lecithin plus SH. For in both cases strong inhibition is obtained with potassium ferro-cyanide, which, as is known, precipitates the copper ions. Indirect proofs can also be found for the fact that other SH systems contain traces of catalytic metal, although probably not copper. Therefore the theory of thiocyanide formation from KCN may certainly be abandoned for the sulphydryl oxidation as well as *a fortiori* for cell respiration.

We must not overlook the fact that the reactions discussed here represent a limited chapter on autoxidation in the cells and only a share towards the solution of the problem of instability of the nutritive substances in the body. It is certain, however, that only the collection of a large number of data will complete the task of tracing the oxidation mechanism in the cell back to the events of inanimate nature.

CHAPTER III

CHEMICAL RELATIONS BETWEEN RESPIRATION AND FERMENTATION

The older physiologists, to whom the unity of all processes of life seemed much clearer than to us modern ones, also have pursued the idea that the different kinds of animal and plant metabolism rested upon a common basis. I do not refer here to such fantastic suggestions as that of a gigantic living molecule whose partial decomposition and reconstruction were at the bottom of all metabolism. I am rather alluding to the productive thought of Pasteur,[118] who first embraced the different kinds of chemical breakdown under the uniform viewpoint of energy-production. For Pasteur was the first to recognize that the fermentation of yeast under anaërobic conditions represents a nearly perfect substitute for oxygen respiration. Under these circumstances the yeast cell can not only live, but also grow and multiply. In the first place there is the need of chemical energy for the cell, which can be satisfied in different ways, as seen in the yeast cell, either by an oxidative cleavage of the sugar, or by its oxidation. But this conception seemed even to Pasteur so strange that he weakened its import by considering fermentation as disguised oxygen respiration. He thought that in this process the oxygen of one half of the sugar molecule burns the other half into carbon dioxide. It would be an intramolecular oxidoreduction, in modern terminology.

We shall not pursue this somewhat artificial hypothesis further, but it is probably the origin of the idea of

"intramolecular" oxygen. This oxygen was believed to perform real oxidations in the animal body, just as the molecular oxygen of the free atmosphere, only it was derived from the organic molecules themselves. When the physiologist Hermann had established the fact that the higher animals also could liberate energy in absence of air, that the prototype of all working devices, the muscle, can be active on deprival of oxygen, he transferred the idea of the intramolecular respiration to the animal organism, especially to the muscle. It was even to be a molecule with excess of oxygen contents, a chemical oxygen depository, called "inogen," [42] on the anaërobic breakdown of which should be based the work of the muscle. Even if this thought was wholly erroneous, as we shall see, it originated after all in the instinctive conception that fermentation-like processes are participating in the activity of muscle.

In a slightly different manner, Pfeffer and Pflüger continued Pasteur's idea. They also assumed that the so-called intramolecular respiration was as proper to the higher animals as to yeast, but that it was only the first step of respiration. For under all circumstances the breakdown of foodstuffs was to start with an anoxidative phase. In the absence of oxygen, the metabolites would remain intact, but in the presence of oxygen burn to CO_2 and water. Fermentation would therefore have to be considered as a partial process of oxygen respiration, *i.e.*, its introduction. Also in this form, the hypothesis has not turned out to be correct. Some authors thought it possible to observe in animal tissue, under anaërobic conditions, the formation of larger amounts of alcohol and carbon dioxide. As regards the alcohol, there is no doubt that it is produced by sterile tissues mostly in slight traces, and

that for the rest it is caused by the activity of bacteria.[38] In regard to the carbon dioxide I consider it as a certain fact that, where it occurs in the animal organism in a large amount under deprival of oxygen, it is not produced anaërobically, but is driven off by the lactic acid formed simultaneously.* [101]

But there is after all a correct principle underlying this theory of the older physiologists. For it is true that in chemical respects respiration and fermentation are connected in various ways. In the highly important case of muscle, it can be shown that the breakdown of molecules starts with an anaërobic phase. The fact that it is the metabolism of muscle which shows this resemblance to the fermentation of yeast, is surely no mere chance occurrence. As we shall see, here it is also a question of the decomposition of carbohydrate. This circumstance may be the common bond which connects the metabolism of such exceedingly heterogeneous cells as those of yeast and striated muscle.

In the last lecture some experiments were described on the respiration of killed yeast cells. It was seen that the oxygen respiration ceased when the yeast was washed exhaustively with water, and that it reappeared on addition of the aqueous extract. In a similar manner the oxygen consumption of the maceration juice (Lebedew) vanished on ultrafiltration, and was reëxcited by the addition of ultrafiltrate, or boiled yeast juice. Furthermore, the whole respiration system, consisting of a dialyzable and a colloidal component, can be precipitated

* Fletcher [22, 24, 26, 28] was the first to try to explain the anaërobic output of CO_2 by a displacement by means of lactic acid. The conclusive proof, however, was still lacking, that the output of CO_2 was entirely due to this and not even partly to chemical formation. I could demonstrate this by comparing the contents of carbon dioxide in the isolated symmetrical gastrocnemii before and after the anaërobiosis. The contents agreed, due allowance being made for the measured CO_2 given out in the meantime.

by 85 per cent. alcohol and proves invariably effective after solution.

We will now turn our attention to the dialyzable constituent designated by me as respiratory substance. It is indeed no uniform compound, and nutritive bodies must also be contained in it. But sure signs were found indicating that in this "respiratory substance" a dialyzable, thermostable co-enzyme was coöperating. Indeed, from the chemical behavior resulted a far-reaching resemblance to the co-enzyme of fermentation, discovered by the English biochemists Harden and Young.[31, 33] The thermostability, precipitability, and susceptibility towards alkaline, lead salt, and alcohol coincided. I could indeed prove that we have to do here with a co-enzyme, common to respiration and fermentation.[89, 90] Assuming that it was a co-enzyme of respiration of yeast, it should also be found in animal organs, especially perhaps in such which abundantly convert carbohydrate, as does muscle. This hypothesis was confirmed in a striking manner. First it was found that a boiled extract of muscle, instead of yeast, can reëvoke the respiration of acetone yeast or of the residue of the juice. But it appeared to me as a crucial test, that this same muscle juice also reëvokes the fermentation of the yeast residue which was abolished by the extraction of the co-enzyme. Since no alcoholic fermentation takes place in muscle, but as we shall see, this co-enzyme also assists in respiration, it is indeed clear that the same body can serve both for respiration and fermentation (see Table I). This co-enzyme can be found in different animal organs, in largest amounts, however, in muscle. In the blood serum it is lacking entirely. In all the organs it is accompanied by an inhibiting body of fermentation, which is, however, thermolabile. This body checks the zymase directly, probably by coagula-

tion. For this reason only boiled juices can be used for the reviving of fermentation, never the cold extracts.

TABLE I. THE CO-ENZYME FUNCTION OF MUSCLE EXTRACT.

c.c. CO_2 from sugar by fermentation with:	In hours			
	2	4	16	24
1. 1.5 c.c. maceration juice	6.6	8.4	10.0	10.5
2. 0.6 c.c. washed residue (condensed 3 x)	0.	0.	0.	0.
3. do + 1.3 c.c. boiled yeast juice	3.0	6.0	10.0	10.4
4. do + 1.3 c.c. boiled muscle extract.	2.3	4.5	9.8	11.5

Within recent years a large number of investigators have been working on substances which may be called activators of fermentation. Substances can be gained from various animal and vegetable material which accelerate fermentation more or less. Some authors have connected with it the hypothesis that such substances might be identical with the B. vitamines, soluble in water. Euler,[20, 21] to whom we owe some valuable contributions to this question, has been able to distinguish most of these substances, which he calls biocatalysts, from the real co-enzyme of fermentation. They stimulate fermentation only if a co-enzyme is present besides. But I consider it beyond a doubt that this boiled muscle juice actually contains a co-enzyme of fermentation, and that it is effective when the genuine co-enzyme of yeast is completely lacking.* It is, however, possible that in chemical respects the co-enzyme of muscle differs in some way from that in yeast.

On the other hand, the problem of the chemical nature of the co-enzyme is still unsolved. I found it impossible to replace it by pyruvic acid, although, as we know from Neuberg's discovery,[112] the latter can ferment with-

* Euler has informed me privately that he agrees with this view.

out co-enzyme and is also a strong activator of sugar fermentation. Neuberg believes, however, that a mixture of different keto-acids behaves like a real co-enzyme.[114] This is not yet quite certain.

If we now ask what significance we must ascribe to this co-enzyme in muscle, we may assert in the first place that it affects the respiration of muscle just the same as the respiration of the yeast residue. Also the respiration of finely chopped muscle tissue is checked by exhaustive extraction with distilled water and reëxcited by the addition of the aqueous extract. Similar statements had been made before by the Swiss physiologists Batelli and Stern.[4] To be sure, several of their assertions did not stand the test, but the fact singled out here is consistent and is of great importance for the mechanism of muscle respiration. Here too a dialyzable thermostable substance, a co-enzyme, is required besides the enzyme contained in the solid residue, and here also we have to do not alone with foodstuffs which we remove from muscle by washing. We can even prove that the nutritive substance, lactic acid, needs a co-enzyme for its oxidation. For in minced muscle tissue, where abundant lactic acid is present from spontaneous formation, lactic acid added externally does not increase the respiration. On the other hand, it is also ineffective if the muscle tissue is washed exhaustively with water and the last traces of the "respiration substance" are removed. On slight water extraction, however, where the co-enzyme is incompletely removed, but the acid strongly decreased, the addition of lactic acid raises the respiration about 100 per cent.[93]

The respiration of muscle which we reëxcite by addition of boiled muscle juice to inactive residue is in every

respect similar to the original one and can amount to 50 per cent. of its rate.

As the washed yeast can be reactivated by boiled muscle juice, so the washed muscle can be activated by boiled yeast extract. In this case, however, the oxidation is not only restored but markedly increased above the normal level, varying in some respects from genuine respiration. The CO_2 formation is diminished, and the rate of respiration in distilled water is higher than in phosphate solution. The great susceptibility of the normal respiration to calcium salt has disappeared. Indeed a large part of this oxidation is not due to the co-enzyme of the yeast extract, but to succinic acid and perhaps also to glutamic acid contained in the juice.[93]

Now the common co-enzyme of respiration and fermentation does not by any means form the only bond connecting both kinds of breakdown of carbohydrate with each other. There exists another group of facts pointing still more clearly to common steps in this metabolism. The English biochemists Harden and Young made still another important discovery in the fermentation process, partially explaining the rôle of phosphate in it.[32, 34, 35, 37] They could ascertain that the phosphate is esterified with the fermenting sugar to hexose diphosphoric acid, according to the following formulæ:

(1) $2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_5OH + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2$.
(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2$.

The esterification, as we see, does not form the preliminary stage of the decomposition of sugar. It is not inserted directly in it, but only one molecule of sugar is esterified, while the other one is cleft. We have here a coupled reaction. What chemical meaning this coupling really has is hard to say. In this connection I found[91] that hexose diphosphoric acid works as a catalyst in

the first stage of fermentation. For fermentation never starts immediately after the mixing of sugar and yeast juice, but rises gradually to the maximum. This rise occurs more quickly on the addition of hexose diphosphoric acid and in proportion to its concentration. Since during the fermentation itself hexose diphosphoric acid is formed, this increase of fermentation is caused partly by autocatalysis of the hexose phosphoric acid. According to Harden,[39] the initial delay in fermentation is due to the slow formation of acetaldehyde, which acts as H_2 acceptor in the sense of Neuberg's theory.[115] Therefore, by adding acetaldehyde, the fermentation sets in with full strength without the "fermentation rise." It is not quite clear what rôle is played in this process by the hexose phosphate. In any case, it acts through itself and not through the hexose formed at its decomposition.

Some years ago, the Frankfort physiologist Embden and his co-workers succeeded in discovering a similar hexose diphosphoric acid in muscle.[17] They considered it as the immediate precursor of the lactic acid, as "lactacidogen," as one molecule of hexose phosphoric acid is split up into two molecules of lactic acid and two of phosphoric acid. At the same time they supposed that this lactacidogen was the store from which the muscle draws its entire formation of lactic acid during the anaërobiosis. This anaërobic formation of lactic acid has been mentioned before, but will now be treated more in detail. It is well known that the English authors Fletcher and Hopkins laid the scientific foundation for the biochemistry of muscle, about fifteen years ago, by tracing the formation of lactic acid in muscle under various conditions: at rest, during activity, during rigor, finally also on injury and on mincing.[27] It was found that in the intact muscle under anaërobic conditions lactic acid

always appears, but not in the presence of oxygen. The latter even caused the disappearance of the lactic acid formed anaërobically. Now, the lactic acid does not accumulate in muscle at random, but approaches a maximum. Such a maximum, that of rigor, is obtained with various forms of rigor, by chloroform, heat, or in the "rigor mortis," as well as on the mincing of muscle. Another lower maximum of lactic acid is obtained on fatigue of the muscle by exhaustive tetanization. One essential point, however, was not cleared up in this investigation, *i.e.*, the source of the lactic acid and its fate when it disappears in presence of oxygen. On the basis of Embden's ideas it might appear as if the lactacidogen was the store from which the muscle drew its formation of anaërobic lactic acid. I found out that this theory was not quite correct, but that the glycogen itself is the store of lactic acid.[98] For the glycogen decreases exactly by the same amount as lactic acid is formed, while the quantity of lower carbohydrates does not undergo any perceptible change.

But at the same time important evidence was found that sugar, before splitting up into lactic acid, is linked intermediately with phosphoric acid.[99] For in studying the formation of lactic acid in minced muscle tissue, left to itself under deprival of oxygen, the maximum, described by Hopkins and Fletcher, is really obtained even long before the glycogen contained in muscle is completely decomposed. This maximum may be put off but cannot be avoided, if muscle tissue is suspended in an alkaline buffer solution.[63] Only in one single medium, as I found, can the whole glycogen contained in muscle be converted under normal temperature with perfect certainty into lactic acid, *i.e.*, in a solution of biphosphate. But this is not all. Nobody had ever succeeded in forming lactic acid from carbohydrate, added externally to minced

muscle.* But this became possible without difficulty by adding new glycogen or hexoses to the muscle tissue suspended in a solution of biphosphate, after the preformed glycogen was exhausted. These experiments could be easily explained by the supposition that the hexose phosphoric acid is an intermediate product of the breakdown of sugar. In minced muscle, however, it is decomposed irreversibly on account of the diffusion of the phosphates from the cut-up muscle fibres. By a high phosphate concentration, this diffusion is checked and with it the irreversible decomposition of the hexose phosphoric acid. In this way a continuous resynthesis of the ester from newly split glycogen and inorganic phosphate becomes possible, and finally there is the complete cleavage of the entire store of glycogen and eventually of added carbohydrates.

Another argument in favor of this theory is furnished by the influence of arsenates. Harden and Young [36] established the interesting fact that arsenates strongly accelerate fermentation by stimulating the cleavage of hexose phosphoric acid according to the equation (2). By this the speed of fermentation is increased in the second stage, while being controlled by the phosphate concentration. The correctness of this analysis was corroborated by an exact analysis of this phenomenon.[91] In Fig. 3, Curve 1 represents the fermentation speed without arsenate. In the beginning it is very high but slopes down when the available phosphate is esterified. In 2 and 3 the process with arsenate is represented. The fermentation speed remains here nearly constant. Now arsenate also considerably accelerates the velocity of lactic acid formation in muscle, and, as will be shown, also increases the

* The older observations of this kind were due to bacterial action. (See Fletcher.[23])

respiration of muscle. Its effect is here therefore largely analogous to that in yeast, since in both cases obviously the hydrolyzing enzyme, the so-called "hexose phosphatase," is stimulated.

At first glance this hypothetical share of the hexose phosphoric acid in muscle seems to be different from that in yeast fermentation. For here, as already intimated,

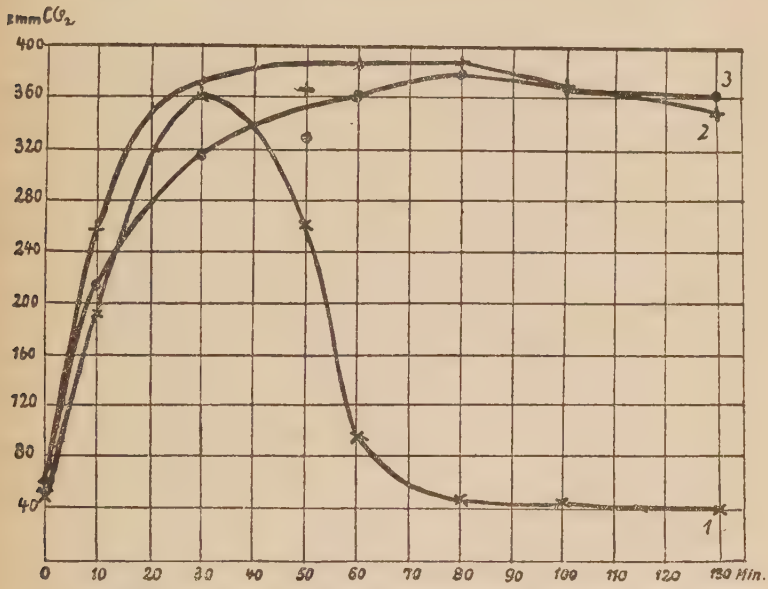


FIG. 3.—*Rate of fermentation of yeast juice under the influence of arsenate.* 1, ×-×, without arsenate; 2, +-+, with 0.005 M arsenate; 3, ●-●, with 0.012 M arsenate.

only one sugar molecule is converted into ester, while the other one breaks down. In the muscle, the sugar is apparently transferred by way of the hexose phosphoric acid into its metabolites. This difference, however, disappears and gives place to a striking similarity when the connection between the formation of lactic acid and respiration is studied more closely. This connection is of great importance because it not only forms the basis

of the resting respiration of muscle, but also of the chemical changes during activity.

If we measure the consumption of oxygen of an isolated muscle at a low temperature for some time, and compare with this consumption the loss of carbohydrate, both quantities correspond with each other within the limits of experimental accuracy. The isolated muscle therefore oxidizes chiefly carbohydrate. If, on the other hand, we keep an intact resting frog's muscle under anaërobic conditions, it continuously develops lactic acid, as we know from Fletcher and Hopkins. Comparing quantitatively the amount of oxygen and lactic acid within the same intervals of time and at the same temperature, we find that the lactic acid cannot be a direct intermediate product of the breakdown of sugar, for there is formed anaërobically about three times as much lactic acid as could be oxidized aërobically with the consumed oxygen. From the law of mass action we could only expect the reverse, *i.e.*, that the accumulation of the acid would delay the process of its formation. But we see that the oxygen prevents a much larger amount of lactic acid from being formed, than would be oxidized by it. If we now put a muscle back into oxygen after a longer period of anaërobiosis, the lactic acid disappears, while at the same time the oxygen respiration is increased above normal consumption. This excess oxygen corresponds approximately with the amount of oxygen excluded by the anaërobiosis. Comparing this excess oxygen with the quantity of disappeared lactic acid, we find that the oxygen has now too caused three to four times as much loss of lactic acid as could have been oxidized by it.

But exactly the same process, only exceedingly increased, is going on during the activity of muscle. If a muscle is working in oxygen, the chemical analysis does

not show anything but the consumption of glycogen, just as in the resting muscle, the intake of a corresponding amount of oxygen and the formation of the equal amount of CO_2. In reality a complex of events is taking place in the active muscle, which can be analyzed more exactly if we let the muscle work anaërobically, either in N_2 or after poisoning with cyanide. We there find the

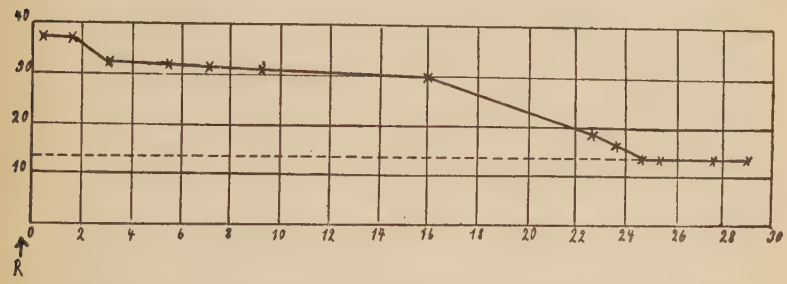


Fig. 4.—*Rate of oxidation during recovery after exhausting tetanization of muscle.* Abscissæ are the time in hours; ordinates, cubic millimeters 0_2 per hour (velocity of oxidation). R, end of stimulation. The full line represents the stimulated muscle; the dotted line the control resting muscle.

following facts: During work just as during rest, lactic acid is accumulated on deprival of oxygen, but in a much shorter time. On the introduction of oxygen to this muscle, the lactic acid disappears, while only about one-fourth as much oxygen is taken in and carbon dioxide formed, as is required for the combustion of lactic acid. Indeed, it has been found that the non-oxidized part of the lactic acid, about three-quarters, is reconverted quantitatively into glycogen.

Fig. 4 gives such an experiment, where the oxidation velocity during the recovery is sketched and compared with the resting value. The total excess oxygen amounts here to 446 cmm. O_2 in 0.7 gm. muscle, or 0.90 mg. per 1 gm. muscle. In the same time 2.57 mg. lactic acid disappeared per 1 gm., wherefrom 0.85 is oxidized.

The significance of this process for the energetics is treated at length in the next lecture. But that we have to deal here with the basic process of metabolism of the muscular system, we see from the fact that it can also be found in minced muscle, suspended in a phosphate solution. Lactic acid develops in it not only in absence but also

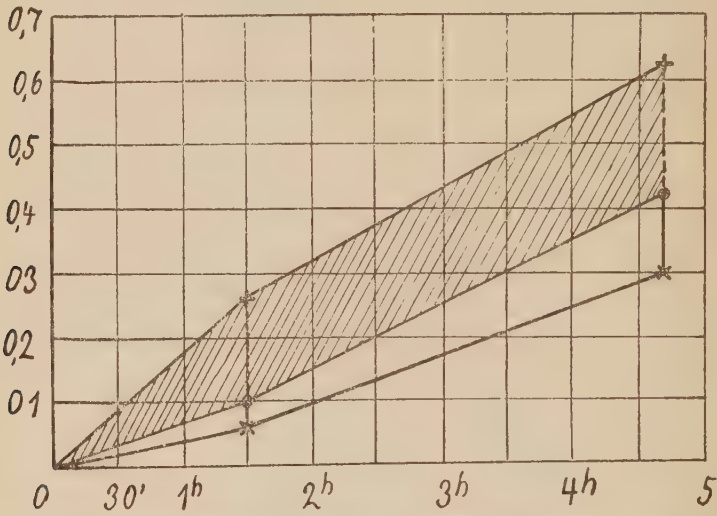


FIG. 5.—*Lactic acid formation in minced muscle in presence and absence of oxygen.* Ordinates per cent. lactic acid. Highest curve (+-+), lactic acid formation in hydrogen. Lowest curve (×-×), lactic acid formation in oxygen; white area between the two lower curves, oxidized lactic acid; shaded area, reconverted lactic acid.

in presence of oxygen, only here much more slowly. Let us now compare in two samples of minced muscle the formation of lactic acid, both during absence and presence of oxygen.[99] If we measure the rate of respiration at the same time, we see that here also the oxygen causes the disappearance of lactic acid in a much larger amount than it could oxidize. This is especially the case during the first hour after dissection. Such an experiment is represented in Fig. 5. The hatched area represents the lactic acid which disappeared without oxidation; the

white area below, the oxidized lactic acid. Almost five times as much lactic acid disappears here as is oxidized. Here also the respiratory quotient is unity. The unoxidized lactic acid has therefore been reconverted into carbohydrate, but, as it seems to me, not completely.*

We therefore notice under different circumstances a disappearance of lactic acid in presence of oxygen, and with it a consumption of oxygen only sufficient to oxidize about one-fourth of the lactic acid. As it is still undecided whether the lactic acid itself is oxidized or its carbohydrate equivalent, the formula given in Table II has been chosen, showing the mechanism of the oxidation of a sugar molecule. The reactions in muscle do not occur exactly stoichiometrically; this is rather an idealization.

TABLE II.

Anaërobic Breakdown.

(1) $5/n\ (C_6H_{10}O_5)_n + 5\ H_2O + 8\ H_3PO_4$
$\longrightarrow 4\ C_6H_{10}O_4\ (H_2PO_4)_2 + C_6H_{12}O_6 + 8\ H_2O$
$\longrightarrow 8\ C_3H_6O_3 + 8\ H_3PO_4 + C_6H_{12}O_6$

Oxidative Recovery.

(2) $8\ C_3H_6O_3 + 8\ H_3PO_4 + C_6H_{12}O_6 + 6\ O_2$
$\longrightarrow 4\ C_6H_{10}O_4\ (H_2PO_4)_2 + 6\ CO_2 + 14\ H_2O$
$\longrightarrow 4/n\ (C_6H_{10}O_5)_n + 8\ H_3PO_4 + 6\ CO_2 + 10\ H_2O$

The process is divided into two separate phases. In the first, the anaërobic, lactic acid is formed from glycogen by way of hexose phosphoric acid, while one molecule of sugar remains unchanged. In the second phase this one molecule of sugar or the corresponding amount of lactic acid is burned. The rest of the lactic acid is reconverted with phosphate to the ester and again becomes gly-

* The amount of reconverted sugar was sometimes less than corresponded with the disappeared lactic acid. The difference exceeded the limit of experimental error.

cogen.* Here we have a coupled reaction similar to the alcoholic fermentation. This second oxidation phase corresponds indeed with the first fermentation equation of Harden and Young. On the other hand, the first anaërobic respiration phase corresponds with the second fermentation equation. As during fermentation, the building up of hexose phosphoric acid is coupled with the breakdown of another sugar molecule, so the synthesis of several hexose-ester molecules would produce in muscle the condition for the oxidation of one sugar molecule, or two molecules of lactic acid. Even if it is not yet firmly established how far we may carry this parallelism of the fermentation and respiration equations, the following points are among those which certainly speak in favor of this theory.

Emphasis was laid before on Harden and Young's statement that the velocity of fermentation depends *ceteris paribus* on the amount of available phosphate or during the longest period of fermentation, on the speed of hydrolysis of hexose phosphoric acid. Corresponding with this I found that the amount of oxygen consumption of muscle depends essentially on the velocity with which the above reaction (1) occurs, the cleavage of sugar into lactic acid. To be sure, the increase of respiration of muscle always presupposes the increase of lactic acid formation, so we may call the lactic acid the "pace-maker" of the respiration of muscle. To this rule I have found no exception. In the intact muscle respiration is in this way increased by electric stimulation. It is also done without stimulation and without visible change of form by con-

* The insertion of hexose phosphoric acid for the resynthesis of glycogen has been chosen here for symmetrical reasons. According to the new facts established by Embden,[18] we must suppose that the inorganic phosphate, set free during contraction, recombines anaërobically with newly split-up glycogen before the lactic acid has disappeared from the muscle. Therefore the reconversion of lactic acid into glycogen occurring under expenditure of oxidation energy, could take place directly without passing through the stage of the phosphoric acid ester.

tracture drugs in small amounts which are not sufficient to produce rigor, *e.g.*, by alcohol, and to an especially high degree by caffein. Exceedingly effective, however, is in this respect the fine mincing of muscle whereby the speed of the formation of lactic acid is increased about fifty-fold, and respiration twelvefold. Now it is very curious that sodium arsenate affects the respiration of muscle tissue exactly as it does the fermentation of yeast juice. For it increases the oxidation, while accelerating the formation of lactic acid. Indeed, it is the most effective substance I have found for it.

This coupling of lactic acid formation with oxidation, according to the two equations discussed above, throws a strong light upon the significance of the resting respiration in general. In most cells which are doing no visible work, we cannot find a fully satisfactory answer to this question. But in muscle the meaning of resting respiration is manifest. Its chemical dynamics are here identical with the metabolism of activity, only its velocity is exceedingly less. For the work of muscle, the explosive formation of lactic acid is the decisive event, while the removal of lactic acid means the recovery. Now, it is evidently easier to obtain an instantaneous effect by increasing a chemical process which is already under way than first to set it going. This increase could be effected, *e.g.*, by an increase of permeability of membranes for the acting substances. We may therefore assume that a certain permeability of the separating interfaces must exist permanently between the reacting bodies, so that an action can be obtained by means of a sudden increase of this permeability. In consequence of this permanent limited permeability, some lactic acid is formed which must be continually removed by oxidation. Respiration at rest is here, therefore subservient to the readiness for

work. We may compare it with the state of a cranked motor car standing still.

To come back once more to the question, what significance might be attached to the co-enzyme common to respiration and fermentation, we may perhaps suggest that it could possibly have a share in the esterification of organic compounds with phosphoric acid. Doubtless some substances become more unstable by such combination.

I succeeded in proving that extracted muscle tissue and yeast preparations cannot oxidize sugar, while they can oxidize hexose phosphoric acid. Muscle tissue shows this behavior in a still more pronounced manner towards glycerol. Of all the glycerol compounds, only glycerol phosphoric acid is oxidized by washed muscle, where inorganic phosphoric acid is split off parallel to the uptake of oxygen. May I make the bold hypothesis that on the one hand the animal body makes fats and carbohydrates accessible to oxidation by combining them with phosphoric acid, whereby they become more labile, and on the other hand, that protein can only burn in cells by being split up into amino-acids? Indeed, amino-acids different from protein burn directly on the surface of charcoal. We may therefore suppose that they are also oxidized on the structural surfaces of the cell. On the other hand, the fats and carbohydrates would need special conditions to render them oxidizable. We should have to count among them esterification with phosphoric acid and also the co-enzyme.

It would give an entirely wrong impression to consider the relations between respiration and fermentation discussed above, as the only ones. Neither could the experiments which led to these conclusions be taken out of their connection with the great number of biochemical works on the breakdown of carbohydrates. Even in

1906, Mandel and Graham Lusk [69] concluded that the phlorhizin-diabetic animal could not only form lactic acid from sugar,[15, 16] but also the reverse, *i.e.*, reconvert lactic acid into sugar in the liver.[15, 16] Chiefly Dakin [13] and Neuberg [113] and also Embden [15, 16] have worked at the stages through which this interconversion of sugar and lactic acid is going on. But I must exclude a certain misinterpretation which some statements of these authors may cause. They seem to assume that a reversible equilibrium exists between sugar and lactic acid, so that the reaction could go spontaneously either in one or the other direction. This is, however, not the case, as will be shown in detail in the next lecture. The cleavage of sugar into lactic acid is a spontaneous process going to completion.* On the other hand the synthesis of sugar from lactic acid requires the supply of energy furnished in the isolated muscle exclusively by oxidation of part of the lactic acid or the corresponding amount of sugar. It can probably be provided also in other organs only by oxidation.

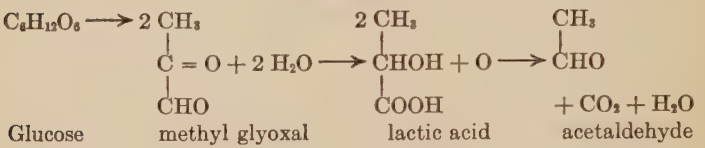
But these considerations do not render the problems as to the intermediate stages of the breakdown of sugar and the way in which lactic acid burns less vital. According to Dakin, Neuberg, and Levene it is not improbable that sugar is decomposed into lactic acid by way of methyl glyoxal. Above all they proved the existence of an enzyme in the animal body which converts methyl glyoxal into lactic acid. Methyl glyoxal, however, according to Neuberg,[111] must also be considered as an intermediary product of fermentation, providing pyruvic

* At 30° and 45°, where the synthetic enzymes of the frog muscle are injured, Laquer [64] found glycogen a better source of lactic acid than glucose. According to him, the reason for this seems to be that only α-glucose, which is the derivative of glycogen, yields lactic acid immediately, while β-glucose first must change into the α-form.

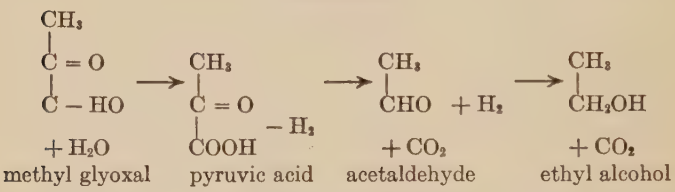
acid. This breaks down into acetaldehyde and CO_2. Finally the acetaldehyde is reduced to ethyl alcohol. In this the transition to pyruvic acid and then to acetaldehyde represents an oxidation, its conversion into alcohol, a reduction. According to recent tests by one of

TABLE III.

Oxidation.



Fermentation.



Neuberg's co-workers [115a] it seems probable that the oxidative breakdown of lactic acid in muscle also occurs by way of acetaldehyde.

We therefore recognize still closer relations between the respiration of muscle and alcohol fermentation. In this lecture have been shown chiefly the relations of both processes from a standpoint which comprises in it the chemical processes underlying the activity of muscle. It may indeed be considered a success of general physiology and its mode of experimenting, that the chemical dynamics of a highly differentiated organ like the muscle could be partly revealed by the study of alcoholic fermentation of yeast.

CHAPTER IV

THE TRANSFORMATION OF ENERGY IN MUSCLE

The problem of the activity of muscle has accompanied physiology, so to speak, from its cradle. This is not astonishing, for the era of physiology coincides with that of technical development. Our science is hardly a hundred years old. In the muscle, nature has produced a machine, so startling and at the same time so perfect, that the explanation of its mechanism could give satisfaction not only to the searching mind, but also promise a rich harvest to the technical progress of mankind. And this problem is so clearly a physical one that even the vitalist admits the possibility of its being solved by means of inorganic natural science. For it is nothing else but the question, how chemical energy in the animal body is transferred into mechanical work. As a fact, the Heilbronn physician, Robert Mayer conceived the law of the conservation of energy first from work of the human body.[72] Helmholtz demonstrated with the thermopile, constructed by himself, the relation between heat and mechanical response of the muscle.[41] At the same time, the chemists were searching for substances which might serve as "the source of muscular energy." Here, therefore, at a decisive point of physiology, the exact physical view first asserted itself against the claims of vitalism.

Within the last decades, however, interest for our subject lagged again. The most productive and progressive minds were those who first got tired of the matter. No wonder, for on the one hand by studying the muscle

exclusively from its physical qualities, it had been overlooked that we have to do with a chemodynamical machine. The comprehension of the mechanism could therefore only be arrived at by the combined efforts of chemistry, energetics, and physics. On the other hand, the biochemist who analyzed only the dead material was helpless in view of the progress of dynamics. Thus experimental analysis stagnated, going round in a circle, and all the theories of muscular function were condemned to fruitless speculation.

Only fifteen years ago the work of Fletcher and Hopkins from the Cambridge Laboratory,[27] discussed in the last lecture, formed the first bridge between the biochemistry of muscle and its performance of work. They noticed in connection with anaërobic contraction and rigor the appearance of lactic acid, and its disappearance on oxidative recovery. This work was followed by the excellent investigations of A. V. Hill [47, 48, 57] "on heat production in muscle," and a series of further studies from Germany and England. Finally the transformation of energy in the working muscle was explained in its chief features.

Since Hermann, it has been known that the muscle can work even if deprived of oxygen, and on complete inhibition of oxidation by poisoning with cyanide. The latter statement was confirmed in a striking manner by the investigations of Weizsäcker [159, 160] on the heart of the frog, where the rate of the work amounted to 60 per cent. on complete stopping of the oxygen consumption by cyanide. This discovery was in curious contrast to the undeniable fact that in the end the combustion of foodstuffs supplies the energy for the muscular work. The connection between oxidation and work could therefore only be quite indirect. The first light was thrown upon this by

the investigations of Hopkins and Fletcher, who found out that the muscle accumulates lactic acid anaërobically on electric stimulation. This goes on until a maximum is reached when it is no longer excitable, whereupon the lactic acid formed can disappear in oxygen and the muscle regains its excitability. This relation immediately gave rise to a series of new questions, starting with that of the maximum of lactic acid. The English authors themselves were inclined to explain this maximum on the following grounds: There exists a precursor of lactic acid of a limited amount; its breakdown causes the fatigue of muscle, and its restoration the recovery. This restoration, they supposed, occurred through the reconversion of lactic acid into its precursor. To be sure, oxidations were to have their part in it, but only for the supply of energy. Lactic acid itself was not to be the fuel, but only the lubricating oil, or another part of the machine, and its presence was considered as being outside the energetic metabolism. This whole supposition was founded chiefly on the observation that the same rigor maximum was obtained, regardless of whether the muscle was put directly into the state of rigor, or after it had been repeatedly fatigued and had again recovered in oxygen. Thus a definite amount of a reconvertible precursor of lactic acid seemed to be present in the muscle. This whole theory, however, was outstripped by later investigations. On more delicate analyses of these relations another picture presented itself. In the first place, the maximum of fatigue did not show itself so constant as Fletcher and Hopkins had assumed. That it fluctuated according to the nutritive conditions of the frogs and the temperature of stimulation, could still be explained by the supposition that under these circumstances the amount of the available precursor changes. But this explanation did not

account for the circumstance that on fatigue with single induction shocks a maximum is reached 50 per cent. higher than with tetanic stimulation. Finally I found convincing evidence for the fact that this maximum does not depend on the existence of a limited amount of precursor, but on the accumulation of lactic acid in the interior of the muscle itself. The obtainable maximum of lactic acid is considerably increased if the isolated gastrocnemius of a frog is stimulated to fatigue in a Ringer's solution containing more alkali and carbonate than usual, resulting from the addition of a mixture of sodium carbonate and sodium bicarbonate. This maximum may now amount to as much as 0.5 per cent. of lactic acid, in reference to the weight of muscle, instead of 0.34 per cent. At the same time a large part of lactic acid has passed into the surrounding solution. The amount in the interior of muscle is changed little. That the quantity of precursor is of no importance here is also seen from the fact that the contents of glycogen decreases in proportion to the lactic acid formed, while the amount of lower carbohydrates does not change. Even on complete fatigue, the supply of glycogen is not yet exhausted.

If we increase the fatigue maximum considerably in the alkaline Ringer's solution, thus removing the lactic acid, we increase simultaneously the whole work which a muscle can perform anaërobically. A gastrocnemius of 1 gm. in weight and 30 mms. in length can in this way develop totally as much as 160 kilograms of isometric tension, while under ordinary conditions only 120 kilograms are produced when we sum up all the single isometric twitches to total fatigue. I named this ratio isometric coefficient of lactic acid $\left(\frac{\text{gm. tension}}{\text{mg. lactic acid}}\right)$. For I found as a general rule that a fixed relation exists under

normal conditions between the lactic acid formed upon stimulus and the developed isometric tension. This fixed ratio, which is also independent of the temperature, assuredly proves that the production of lactic acid is connected with the mechanical response. But while the unfatigued muscle with a definite amount of lactic acid always develops a proportional amount of tension, the efficiency of lactic acid is lowered with fatigue and also with incomplete narcosis. This seems important for the elucidation of the more delicate events in the contractile mechanism.

A still larger problem is connected with the disappearance of lactic acid in the oxidative recovery. Hill recognized the principle very accurately indeed when he compared the activity of muscle with the function of an accumulator.[45, 47] The electric energy which a charged accumulator delivers on closing the circuit originates in the end in the supply of energy in being charged. According to Hill, this charging is done in muscle during the recovery period, when by the expenditure of oxidations potential energy is accumulated. A certain amount of this is liberated on stimulation during contraction, just as in an accumulator on closing the circuit. Taking another simile, we may compare the working of muscle with a clockwork. In the recovery period the clock is wound up. Each stimulus liberates one stroke, and the single strokes are not distinguished from each other until the clockwork has run down. The cause for its having run down is clear to us already; it is the accumulation of lactic acid. But in what does the winding up consist? To be sure, in the removal of lactic acid. But as to how this is going on, opinions have differed for some time. Hill has derived his conception of an accumulator from his fundamental discovery that the heat of muscle is not

evolved simultaneously with the contraction but in two phases of approximately the same amount—the first, so-called "initial heat," during the twitch; the second, so-called "delayed heat," during the oxidative recovery. The latter, however, appears chiefly in oxygen only, and Hill justly connected it with the oxidative disappearance of lactic acid. He went even further and reasoned from his measurements and those of his co-worker Peters,[46] that this disappearance of lactic acid could not be attributed to its complete burning up. For in some not quite conclusive experiments the authors had also measured the heat produced by the formation of lactic acid on rigor and anaërobic stimulation and calculated it at about 450 cals. per 1 gm.[44, 119] If in the recovery period about as much heat would be evolved, there would result 900 cals. per 1 gm. of lactic acid. The combustion heat of lactic acid, however, is more than 3600 cals., so here 1 gm. of lactic acid cannot burn completely. Nevertheless, this theory was finally adopted by the English investigators for some time,[29] for Hill's and Peters' measurements were not quite exact and on the other hand Parnas,[117] also in the Cambridge Laboratory, thought to have proven that consumption of oxygen and disappearance of lactic acid in the recovery period corresponded with each other. Indeed, it was very difficult to make Parnas' results accord with those of Hill and Peters.

In reality, however, the relation is a different one.[92, 98, 104] It is true that after the anaërobic stimulation the oxygen intake is strongly increased for a definite period. This increase is closely connected with the disappearance of lactic acid, and the intake of oxygen above the resting value, which we may call "excess oxygen," only keeps on as long as lactic acid can be proven in muscle. But the total amount of this excess oxygen is only sufficient to oxidize about one-fourth of the disap-

peared lactic acid. As a matter of fact, the rest, *i.e.*, three-quarters, are reconverted quantitatively into glycogen. Therefore in this recovery period a process is going on which we know already as partial process of muscle respiration. The other part, however, the reconversion of glycogen into lactic acid, belongs to the working phase.

The significance of this coupling between oxidation and reconversion can be understood completely from the energetic standpoint; 1 gm. of lactic acid is produced from 0.9 gm. of glycogen. The combustion heat of glycogen amounts to 4191 cals. according to Stohmann. Emery and Benedict stated higher values;[19] but an accurate determination of the combustion heat of glycogen, in Mr. Roth's Institute at Brunswick, Germany, made upon my suggestion, completely confirmed the correctness of the old value of Stohmann.[121] It was 4188 cals., *i.e.*, 3770 cals. per 0.9 gm. The combustion heat of lactic acid should be 3661 cals. according to Luginin. I found, however, 3601 cals. in a new determination by means of zinc salt, for diluted acid, with which we are concerned here.[102] The repetition in Mr. Roth's Institute brought forth exactly the same value (average 3603 cals.). After the work was finished, Prof. Hill drew my attention to the older determination by Emery and Benedict, which amounted also exactly to 3601 cals. for diluted acid.[109] Therefore the difference between the combustion heat of glycogen and diluted lactic acid amounts to almost 170 cals.* If, now, nothing else would

* This calculation would be changed considerably, however, if the latest statements of Slater[124] concerning the combustion heat of glycogen from sea-mussels would be confirmed on muscle glycogen from vertebrata (frog or rabbit). According to Slater there exists a definite glycogen hydrate with the empirical formula $(C_6H_{12}O_5)_n$, which gives a combustion heat of 3883 cals., 110 cals. more than the value of Stohmann and Roth-Ginsberg, calculated for the same formula. In this case some of the following arguments had to be modified, as no "gap" of unexplained calories would remain.

take place but the evolution of lactic acid from glycogen during the working phase and its oxidation during the recovery period, then only 5 per cent. of the energy would be liberated during the working period and 95 per cent. during recovery. This would make an impossible machine. But the process is entirely different. In the first place, I found in a large number of determinations, that in muscle, on the formation of 1 gm. of lactic acid, not 170 cals., but 380 are liberated. This number is not perfectly accurate on account of the great technical difficulties of measurement. But since it represents the mean of numerous determinations without systematic errors, it may be wrong by only a few per cent. As far as accuracy goes, the heat formation is the same under different temperatures. Before inquiring into the source of the excess of 200 cals. above the difference of the combustion heat, the energy balance of the recovery period will be calculated first.

As is shown in Table II of the last lecture (p. 55), and has been proven by my experiments, about 4 molecules of lactic acid disappear when one is burned; then $3772/4 = 943$ cals. must result for 1 gm. of reacting sugar (= 0.9 gm. of glycogen). Since we have determined about 380 during the working period, the rest, *ie.*, 560 must be looked for during the recovery. Accordingly, 40 per cent. of heat would appear during the working phase and 60 per cent. during recovery. I could establish this very well, at least in the order of magnitude, with a technique first used by Parnas. By direct measurement of the total heat of recovery in a fatigued muscle and comparing this heat with the consumption of oxygen I found: 1, that the heat of recovery formed above the resting value was about the same as the heat of anaërobic fatigue; 2, that this heat, in relation to the intake of

oxygen, was less than corresponds to the combustion of carbohydrate. For 5 cals. per c.c. O_2 must be formed on the combustion of carbohydrates. But on the average of the recovery period there were only 3.5 cals. per c.c. O_2, and in all exactly as much heat was lacking here as had appeared during anaërobic fatigue.

We will now compare this result with that which Hill,[45] and later on Hill and Hartree,[52] have obtained by direct myothermic measurements. As mentioned before, in Hill's previous works it had been stated that the proportion of initial to delayed heat is about unity. Therefore this agrees with our results. But a still more accurate comparison is now of interest. For, as can easily be seen, the proportion of restitution heat to fatigue heat must be given by the quotient of the disappearing molecules of lactic acid to the oxidized ones. In an ordinary coupled reaction, taking place in a homogeneous system, this proportion should be constant. But muscle consists of a heterogeneous system, and for this very reason we cannot expect such a fixed stoichiometrical relation, which does not exist either. Indeed, I found, for instance, in minced muscle that evidently only one of five molecules of lactic acid is oxidized. Thus $3770/5 = 754$ cals. would belong to the exchange of 1 gm. of sugar. Upon the restitution phase devolve therefore $754 - 380 = 374$ cals.; the proportion of restitution heat to fatigue heat would be 1. In the same way it is found that, if only one of six molecules of lactic acid is burned, this proportion would be 0.63 : 1. The more molecules of lactic acid can be reconverted by the oxidation of one single molecule, the better the machine is working. Therefore this figure gives the scale for the efficiency of recovery, it expresses how much of the oxidation energy is used for endothermic reactions, for the charging of the accumulator. From our measure-

ments there followed an efficiency of 40 per cent., while it would be 60 per cent. in the case discussed last. There is no doubt now, after comparing Hill's figures with mine, that the efficiency is better the less tired the muscle is, and the more quickly it can recover. In this point the myothermic technique has an exceedingly great advantage over the calorimetrical and chemical ones. Hill and Hartree obtain an average efficiency of 52 per cent. for the recovery, to which corresponds a proportion of 5.2 molecules of disappearing lactic acid to 1 molecule oxi-

Table I. Balance of the Recovery.

l. a. disappeared / l. a. oxidized	4/1	5/1	6/1
Total heat per 1 gm.	943	754	630
Recovery heat per 1 gm.	560	374	250
Oxidative heat / Anaërobic heat	1.5:1	1:1	0.63:1

dized. We may therefore correctly assume that in the optimum even 6 molecules of lactic acid may disappear by the combustion of 1, which would mean an efficiency of recovery of 60 per cent.

Hill's and Hartree's results could not be gained except on the basis of a more delicate analysis of the time course of anaërobic heat.[48, 49, 52] With brilliant skill the two English investigators succeeded in analyzing accurately the time course of heat in isometric contraction per 0.1 second, and could even distinguish four different anaërobical phases. Three of these belong to the contraction itself, and may be designated together as initial heat. A certain part of this heat is liberated during development of tension, another part during the state of tension, and the third part during relaxation. (Fig. 6.) This relaxation heat, which may form as much as one-third

of the entire heat, is very interesting. Its physicochemical significance will be touched upon later. In the present connection the fourth phase is of special interest to us. This phase does not belong to the initial heat, but is a delayed heat. It is related to the partial recovery which the muscle shows after contraction even in absence of oxygen. We may consider it as an anaërobic restitution

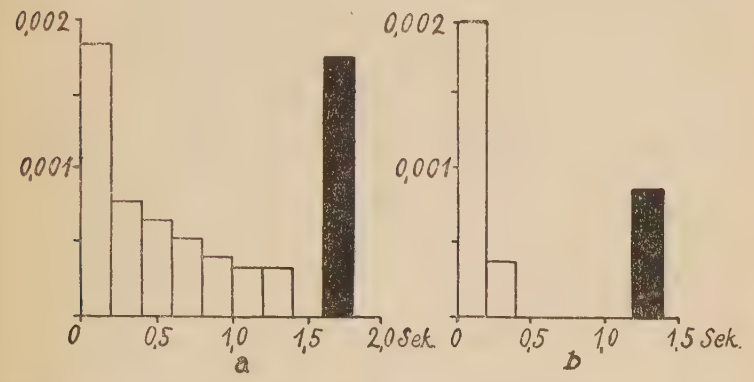


FIG. 6.—*Time curve of the initial heat analyzed per 0.1 sec. according to Hill and Hartree. a*, long stimulus; *b*, short stimulus. The black area represents the heat of relaxation.

heat, according to Hill and Hartree. It corresponds to 30 per cent. of the initial heat. Therefore under anaërobic conditions the proportion of the initial heat to restitution heat is found to be about 1 : 0.3. In presence of oxygen, however, it was found to be 1 : 1.5. Since the anaërobic restitution heat, whatever its share may be, must also be contained in the oxidative restitution heat, there follows from it. If we fix the initial heat at 1, the heat caused by oxidation amounts to $1.5 - 0.3 = 1.2$, and the total anaërobic heat $1.0 + 0.3 = 1.3$. Based on this calculation, we obtain, as do Hartree and Hill, the following relations, taking as standard the value of 380 cals. for formation of 1 gm. of lactic acid. (Table II.)

We now turn to the question, What chemical and physicochemical processes are happening during the contraction act? We must first of all consider the fact that under all circumstances, even in oxygen, lactic acid is formed and only disappears after the contraction has ceased. This fact, important for any theory, is proven by the exactly equal rate of initial heat in the absence and presence of oxygen, and by the slow rise of the oxidative

TABLE II. HEAT-PRODUCTION IN THE LIBERATION AND REMOVAL OF 1 GM. OF LACTIC ACID IN A MUSCLE IN OXYGEN.

Phase.	Relative value.	Absolute value calories.
Initial anaërobic	1.0	295
Delayed anaërobic	0.3	85
Total anaërobic	1.3	380
Total delayed heat	1.5	440
Delayed anaërobic	0.3	85
Difference, delayed-oxidative	1.2	355

recovery heat, several seconds after the relaxation is over. This is easily understood because the chemical process during activity corresponds here with that during rest. The slow rise towards a maximum is probably caused in this way: The lactic acid, formed during contraction, only gradually increases the resting respiration to the height of recovery oxidation.

The question, What is happening during the contraction act? must be connected with the analysis of the contraction heat which we have measured at 380 cals. per 1 gm. of lactic acid. The difference between the combustion heats of glycogen and lactic acid amounts, as stated before, to only 170 cals. per 1 gm. How does this discrepancy come about? I found out the following facts:[101] In comparing the formation of heat and lactic acid with each other, not in the working muscle but in the minced

muscle tissue suspended in a phosphate solution, we obtain pretty exactly 200 cals. per 1 gm. instead of 380. At the same time the lactic acid passes into the phosphate solution. Now, the neutralization heat of lactic acid with biphosphate amounts to 19 cals. per 1 gm. To this is added the heat of cleavage of glycogen into lactic acid, equal to 170 cals. This makes in all 190 cals., which agrees within the limit of experimental error with the value measured, *i.e.*, 200 cals.

Now it can be shown that also in the intact muscle the heat is diminished in the same degree when the lactic acid can escape from it. We first compare the formation of heat and lactic acid during the resting anaërobiosis in unskinned frog's limbs at short intervals. We find the heat here the same as on activity, *i.e.*, 380 cals. per 1 gm. of lactic acid. Indeed, the initial and final conditions of the muscular system are here exactly the same as in the working muscle. The heat therefore agrees in both, although all intermediary links connected with the contraction are lacking. But the heat is different in skinned frog's limbs kept for some length of time in an alkaline carbonated Ringer solution. During the time of the experiment about 50 per cent. of the lactic acid passed into the surrounding fluid. In this case, only 280 cals. per 1 gm. of lactic acid were formed, the heat production decreased from 380 calories at the beginning to 220 cals. at the end, corresponding to the gradual passing of the acid into the Ringer solution. (Fig. 7.) The special evolution of heat, however, which the lactic acid produces in the intact muscle, if remaining in the tissue, has been proven to be due to the hydrogen ion. If we allow an acid to penetrate into a living muscle from the outside—I used valerianic acid—we obtain without any formation of lactic acid a heat of the same order of magnitude as is

produced by the reaction of lactic acid with the substance of living muscle.

What is now really the cause of this heat? It is due for the largest part to nothing but the peculiar manner of the buffering of lactic acid in the muscular tissue. For,

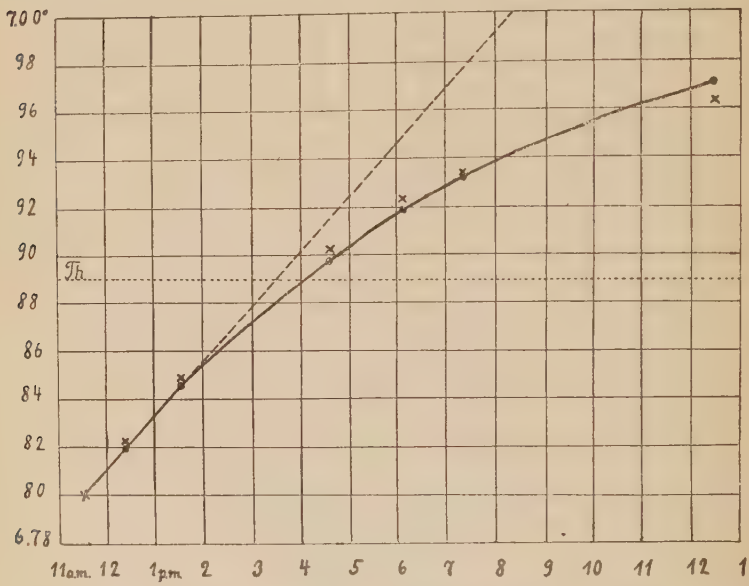


FIG. 7.—*Heat production of skinned frog legs in alkaline Ringer solution.* ×-×, measured temperature rise; full line, corrected temperature rise; dotted line, theoretical value of temperature rise with constant heat formation. The slope of the curve corresponds to the diffusion of the lactic acid from the muscle into the solution.

as can be shown by calculation, the amount of phosphate and carbonate in muscle is quite insufficient to neutralize the accumulated lactic acid to the extent to which neutralization actually occurs. The real buffer, however, which neutralizes the hydrogen ions of lactic acid is the tissue protein. In this buffering there occurs an unionization of protein. A heat is produced which may be called "unionization heat;" that means the reverse dissociation heat of protein. This heat, which could be found with the free

amino-acids as well as with isolated protein, is of exceedingly large amount. We first prepare a solution of glycocoll with caustic soda, *e.g.*, a glycocoll mixture according to Sörensen's technique. Then a small amount of hydrochloric acid or lactic acid is added and the reaction will take place as given in the formula.

$$Na^+G^- + H^+L^- = Na^+L^- + (GH) + 11{,}000 \text{ cals.}^*$$

The hydrion concentration hardly changes at all during this process. From the completely dissociated sodium glycocoll is formed the weak undissociated acid glycocoll, and during this reaction we measure a molar heat of a little over 11,000 cals. This is nothing else but the reverse heat of dissociation of glycocoll. We now buffer in the same way a concentrated solution of protein, free from basic salts, by addition of caustic soda to about pH 8.** Then we add lactic acid in such an amount that the H concentration hardly changes. Thus the reaction is evidently occurring as represented in the equation:

$$Na^+ P^- + H^+ L^- = Na^+L^- + (PH) + 12{,}600 \text{ cals.}^{***}$$

This reaction leads to a still greater heat evolution, with the protein of muscle in presence of ammonium salts, amounting to 12,650 cals. per molecule. By a series of arguments, the hypothesis could be supported that we have also to do in this case with a dissociation heat. There is the rule that the difference between the neutralization heat of an acid and its dissociation heat is equal to the well-known dissociation heat of water, *i.e.*, 13,700 cals. This is exactly the case here. Adding caustic soda instead of hydrochloric or lactic acid to the protein buffer to pH 8, we obtain the neutralization heat of the weak protein acid which, subtracted from the negative dissocia-

* G: glycocoll anion, L: lactic acid.

** Concerning the formation of protein-salts see Loeb's book[65a].

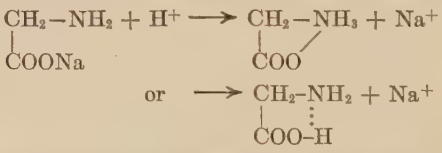
*** P: protein anion.

tion heat, gives exactly the dissociation heat of water. (See Table III.) The negative dissociation heat of protein found here represents the largest dissociation heat of any acid known at all. It might be due to the fact

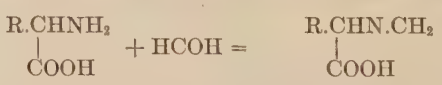
TABLE III.

Protein.	Addition.	Heat per mol.	Therefrom heat of dissociation of water.
Albumin.....	Lactic acid NaOH	−12,370 + 1,370	13,720
Muscle protein.....	NaOH Lactic acid	+ 1,080 −12,600	13,680
Muscle protein.....	NaOH Lactic acid	+ 1,025 −12,700	13,725

that on the unionization of amino-acids and protein there is taking place a formation of an internal ammonium salt, or at least a linkage of hydrogen and nitrogen through partial valencies according to the scheme sketched below.*



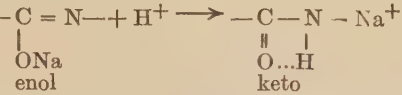
This fact can easily be shown. As is known, the amino-acids react with formaldehyde according to the equation:



* In assuming an internal ammonium salt, the reaction between the amino and carboxyl groups should correspond to the neutralization of a rather strong organic acid with ammonia. We find indeed an ionization heat of the same order of magnitude as this neutralization heat. In alcoholic solution the amino-acids show an increase of the acid and also of the basic dissociation. Löffler and Spiro [67] have therefore assumed that on the formation of the molecule no partial affinities would be saturated in alcohol, as happens in water. But I found that the ionization heat of glycocoll is the same in alcohol and in water. Therefore the supposition of the formation of an internal ammonium salt seems to be the simpler thing. [107a]

The methylene compound thus formed has lost the amphoteric qualities of amino-acids, and at the same time the large negative dissociation heat has disappeared.

In protein probably the changes from the enol form to the keto form occur besides, as has been discussed by Dakin and Pauli.



The dissociation heat of 12,600 cals. yields 140 cals. per 1 gm. of lactic acid. We learned before that of the 380 calories of anaërobic contraction, 170 are due to the splitting up of glycogen into diluted lactic acid. There remain 210 cals., of which 140 can be explained by the unionization of protein. Only 70 cals. remain unexplained. I suggest that this too may finally be traced to the dissociation of protein, and that peculiar conditions, still unknown, are responsible for the increase of dissociation heat of protein in muscle. If this is correct, the entire anaërobic heat would be due to the cleavage of glycogen into lactic acid, to the dilution of lactic acid, and its interaction with alkali protein. We could then boldly assert that the capacity of muscle for the performance of anaërobic work, dealt with in the beginning, depends on the alkali combined with protein, the amount of which would determine the height of the fatigue maximum of lactic acid.

We may now ask whether these results can be used for the explanation of the four phases of anaërobic heat production, as found by Hartree and Hill. Some indirect points could be found, especially from the comparison of the temperature coefficients of the single contraction phases with the chemical processes, indicating that the

liberation of lactic acid occurs during the moment of shortening, while the interaction of the lactic acid with protein belongs to the relaxation.

According to Hill and Hartree, the development of tension has a temperature coefficient of 2.5 per 10°, the relaxation, however, one of 3.6.[50, 51] Corresponding to it I found a temperature coefficient of 2.4 of the lactic acid formation in minced muscle, where besides the chemical reaction only the ionic interchange occurs. But in the intact muscle, where the formed acid reacts with protein, we have a coefficient of 4.[101] This increase of the coefficient might therefore be brought into relation with the interaction of muscle protein. Hill's relaxation heat mentioned before must indeed be imagined as having been produced by a superposition of different chemical and physical phenomena and is finally due to that part of the total energy of contraction which has been transformed into mechanical or elastic tension. However, the unionization of protein evidently forms its basis. We thus obtain a lucid picture of the relaxation process; it depends upon the depression of acidity in the "shortening places" of muscle. In reversed manner we shall make the hydrions responsible for the onset of shortening.

On the other hand, it is not possible for the present to find a satisfactory explanation for the anaërobic restitution heat, the existence of which can no longer be doubted after Hill's and Hartree's measurements. I have proposed to explain it by the unionization of protein, progressing in stages. I think that the protein immediately adjacent to the shortening places could be again dissociated by accepting alkali from other more distant proteins with stronger dissociation heat. A certain restitution would be made possible by it. We must, however,

not overlook the fact that we have to deal here with an hypothesis made *ad hoc*.

However, one general point of view seems to be of interest here. The muscle machine itself consists doubtless of protein, while carbohydrate represents the fuel by the burning of which the machine is actuated. Somehow, the oxidation energy of the fuel must take part in the mechanism of the machine itself. With this unionization of protein we have found a point where this is actually the case. In consequence of the coupling of oxidation with the resynthesis of lactic acid, the endothermic restoration of glycogen is accomplished. Moreover, what is still more necessary for the activity of the machine, the alkali is again set free, which causes the endothermic involuntary dissociation of the proteins. The charging of the accumulator, according to Hill, the storing of potential energy, is therefore to be looked for in the conversion of lactic acid into glycogen and in the restoration of the alkali protein.

Doubtless the great dissociation heat of proteins plays in other ways too an important rôle in the animal body. Hill and Brown [54] recently discovered that with its help the reaction heat of carbonic acid in the blood can be perfectly explained. The process occurs in this way:

$$H^+HCO_3^- + Na^+(Hb)_n^- \longrightarrow Na^+HCO_3^- (H(Hb)_n) \quad (Hb_n = \text{Hemoglobin})$$

The process is accompanied by the evolution of 12,100 cals., of which 11,500 must be counted as belonging to the dissociation heat of the weak protein acid $H+(Hb)_n^-$. There is no doubt that wherever carbonic acid and other acids appear in the tissue, and where protein is working as a buffer substance, this reaction heat obtains. In reversed manner conclusions can be drawn from the measurement of this heat on the condition of protein

in tissue. But the pursuance in this direction would carry us too far from the subject in hand, namely, the energetics of muscle.

These investigations have not only a theoretical interest, but also give us practical explanations about muscular exercise of the healthy and the sick human body. As an example I mention only the practical application which Hill and Lupton [53] have recently made of our various results in basing upon them an exact physiology of exercise. They studied on themselves the consumption of oxygen and the output of carbon dioxide during steady, very violent exercise, especially during flat running. They found that with increasing speed of running, the consumption of oxygen and the output of carbon dioxide first increased to very high values. The respiratory quotient remains exactly unity, corresponding to the exclusive combustion of carbohydrate. But finally a limit is reached, above which the intake of oxygen cannot possibly rise. This is about 4 L. of oxygen per minute. It is, however, possible to increase the speed beyond this limit for a short time. If they determine the respiratory exchange after such exhausting effort, they find for several minutes the intake of a certain amount of "excess oxygen" beyond the rest values before and after. The total amount of this excess oxygen enables us to calculate the quantity of lactic acid which was accumulated at the moment when strength gave out. We assume with Hill and Lupton for the healthy man the most favorable efficiency of recovery oxidation, where the burning of 1 molecule of lactic acid is combined with the removal of 6 molecules *in toto*. In this way we find, in an extreme case, in an athlete of 72 kgm. of weight, 107 gm. of lactic acid accumulated, which, with an approximate muscular weight of 25 kgm., correspond

to an amount of 0.4 per cent. of lactic acid in muscle. This agrees with the highest values I have found as maximum in the isolated gastrocnemius of frog and guinea-pig. The study of the output of carbon dioxide is also interesting. As soon as the excess oxygen exceeds a certain amount, the respiratory quotient rises above unity and can reach values up to 2.6. It is caused by the driving off of carbon dioxide by lactic acid. In this way can be established how large a part of lactic acid decomposes carbonate, and how large a part combines with protein. I calculate that in an experiment of the author's, on an accumulation of 58 gm. of lactic acid, 8 gm. of carbon dioxide were driven off, while 50 gm. must have interacted with protein. For theoretical reasons it is probable that the efficiency of energy-exchange considerably decreases as soon as the lactic acid drives off CO_2 from the blood, instead of unionizing the proteins. Also the ratio $\frac{\text{removed lactic acid}}{\text{oxidized lactic acid}}$ in the recovery state in man could be determined by indirect methods in Hill's laboratory by Long and Lupton.[55, 56] During and immediately following severe prolonged exercise, lactic acid enters the blood, displacing CO_2 from bicarbonate. During the later stages of recovery from exercise, when bicarbonate is practically the only buffer restored, the CO_2 retained in any interval gives a measure of the lactic acid removed, and the O_2 used in excess of the resting value, a measure of the oxidized lactic acid. The average ratio determined in this manner is about 4 : 1, the same as in my experiments on the frog. Higher values with an average of 6.6 were found on calculating the removal of lactic acid on analyses of venous blood. But this method seems to be evidently less accurate

because the lactic acid is probably not uniformly distributed throughout the water phase in the body, as the authors must assume.

It is doubtless very interesting to continue working in this line, studying more intensely the efficiency of muscular work under the circumstances mentioned, the buffer capacity of man, and other points in connection with it.

CHAPTER V

THE ENERGETICS OF CELL PROCESSES

We may consider it as an established fact that the metabolism can be regarded from two standpoints, both of which are valid, *i.e.*, from the energetic and from the purely chemical one. Within recent times the latter has come more to the front as a reaction against the partiality of which the defenders of the purely energetic or caloric theory of metabolism had been guilty. Today nobody can deny that it is altogether wrong to lay down a schematic rule, let us say for the human being, which considers in nutrition exclusively the requirement of calories and the protein minimum.

In connection with this subject I state at once that even where the foodstuffs are required most directly for the accomplishment of mechanical work, *i.e.*, in muscle, it is wrong to speak of an "isodynamy," an equivalence in regard to their heat of combustion. I hereby leave aside the circumstance that for transformation into mechanical work not the so-called "total energy" liberated as heat must be considered, but the "free energy" which may totally differ from the former. We may assume that just in the combustion of animal foodstuffs the difference between the two magnitudes is not very great. On the other hand it seems to me an essential point that, as I could show on the isolated frog muscle, the muscle uses exclusively carbohydrate for the performance of work, that the lactic acid, which is the real working substance in muscle, is derived only from carbohydrates. If on exclusive nutrition with fat or protein the body can never-

theless perform work, it is presumably only done by its capacity of transforming first, protein and fat into carbohydrate. A difficulty was seen in the fact that the "isodynamic equivalence" of the nutritive substances for the performance of work is not complete but still it is far-reaching, while during the conversion of fat into carbohydrate a great loss of energy would occur. Indeed, of 1.6 cals. of fat only 1.0 cal. of carbohydrate is left, and 0.6 cal. would be liberated during the oxidation of fat into carbohydrate. On the other hand, Atwater [3] found in the oxidation of fat instead of sugar only a decrease of 5 per cent. in the efficiency of muscular work of man, and recently Krogh [61] found a decrease of 10 per cent. But we must not overlook the fact that we have to do here with warm-blooded animals, and that the presumed oxidation of fat into carbohydrates saves other heat formation in the body. If these experiments could be made on cold-blooded animals, the efficiency of fat for the work of muscle would doubtless be very much less than that of carbohydrate. (A number of scientists, among them Graham Lusk,[68] think that fat is not changed first into carbohydrate, but can furnish directly the energy needed for the work performed. Indeed, this is not impossible for the living animal, although not very probable. Only by further experiments can the pro and con of this matter be decided.)

Apart from the special mechanical work of muscle we will discuss here the energetic significance of metabolism in general; first the subject of respiration as a whole and then single definite cases where chemical work is produced in the cell, especially on the assimilation of carbonic acid and nitrates. My own work has been a closer study of the chemical assimilation of carbon dioxide by the nitrifying bacteria.[82, 83, 84] The assimilation of nitrates

in the green plant-cells has been lately studied by Warburg.[156] By the same author the energetic relations in the photochemical assimilation of carbon dioxide have been explained quite recently.[149, 158]

There is probably no doubt that the different kinds of animal and plant metabolism can be conceived comprehensively only from the standpoint of exchange of energy. Let us think of the bacteria which oxidize the inorganic substances H_2S, NH_3, Fe, etc., during their respiration without taking in organic material. Let us attempt to explain the several anaërobic fermentation processes uniformly; we can do so, notwithstanding all special chemical purposes of the different kinds of metabolism, only by assuming that the cell needs the energy of these chemical reactions. Therefore the energy, which is finally released in the cell as heat, is by no means an unavoidable by-product of the exchange of substances, but the chemical exchange occurs largely on account of this energy. This point of view can also be applied semi-quantitatively upon the different kinds of metabolism. During the fermentation of 1 molecule of sugar into alcohol and carbonic acid, 26 cals. are liberated; during the oxidation of 1 molecule of sugar, 672 cals. The proportion is therefore about 1 : 25. In comparing with it on the one hand the anaërobic growth of yeast cells during the fermentation of 100 gm. of sugar and sufficient nutrition of nitrogen, and on the other hand, the growth of micro-organisms without fermenting power during oxidation of the same amount of sugar, we find the following results according to different authors: 1. During the alcoholic fermentation, the increase of weight amounts per 100 gm. of fermented sugar to about 1 gm. of dry weight, *i.e.*, 1 per cent. of the weight of sugar. On the other hand, during the oxidation of sugar by penicillium glaucum, or asper-

gillus niger, the assimilation amounts to 33–43 per cent. of the converted sugar. This proportion corresponds therefore to the production of energy, twenty-five times increased in the case of oxidation. But this comparison is only possible if we compare the anaërobic growth of yeast with the oxidative growth of microbes without fermenting power, but not with the growth of the yeast cell itself in oxygen. For under these circumstances the yeast would not only respire, but ferment at the same time, although the latter seems unnecessary from the energetic standpoint. (Pasteur [118] was mistaken when he assumed that the yeast ferments the sugar only under anaërobic conditions, but not in oxygen. But such a behavior is shown by mucor mucedo, where the energetic bearing of the fermentation is seen still more clearly.[118]) We must therefore not apply too partially a purely energetic scale. Nevertheless, the example teaches us that the exchange of energy has to accomplish definite purposes for the vital activity of the cell and especially for its growth. It is indeed a fact, as, for example, Jacques Loeb's experiments on the sea-urchin egg prove,[66] that in obligatory aërobic cells the cell-divisions come immediately to a standstill if we deprive them of oxygen, and thereby stop the exchange of energy. In view of this, a number of the older physiologists, Liebig, Pflüger, also Zuntz, had inclined towards the opinion that during the growth a storing of potential energy might take place. For the living protein, produced during the assimilation of food, had to have higher contents of energy than the dead, which is used for nutrition. In different ways I subjected this hypothesis to tests which yielded perfectly negative results. Warburg had already proven that respiration and growth of the cell can be completely separated from each other.[134, 136] If, for example, fertilized sea-urchin

eggs are put into a highly diluted solution of phenylurethane (0.01 per cent.), the cell-division is stopped entirely without any change in the rate of oxidation. If, now, in consequence of the lack of assimilation or of inhibited formation of structures, a certain amount of energy should here have remained unutilized, the heat production of the egg, referring to the same consumption of oxygen, ought to have been greater during the state of inhibited growth than during the occurrence of cell-division. This was, however, not the case.[75] The heat formed upon the unity of oxygen, which I called "calorific quotient" of oxygen, was almost exactly the same in both cases.* In the same way, if the living protein possesses a higher potential of energy, heat ought to be released on the sudden killing of a large number of cells—this does not occur either.[76] I suddenly killed, *e.g.*, in the calorimeter bottle a large quantity of concentrated actively respiring erythrocytes of birds by addition of acrolein, but no perceptible heat was liberated by it. The accuracy of these experiments is sufficient to state that the combustion heat of protein amounting to about 5800 cals. per 1 gm. cannot differ by 0.1 cal. in the living and the dead substance.**

We see therefore that in this way we cannot find an explanation for the necessity of energy exchange in the cell, *i.e.*, of cells which perform no external work. Growth

* This heat had been given by me, with slightly corrected calculation, as 2.85 cals. on the average. But O_2 was here considered as dissolved. For the comparison with combustion heats, where the oxygen is introduced in gaseous form, the heat of solution of the oxygen must be added, so that we obtain 2.95 cals. In a repetition of my experiments, Shearer[123] found a value, which is perhaps more accurate, *i.e.*, 3.05–3.2 cals., by Hill's thermoelectric method. These figures agree approximately with the calorific coefficients of protein and fat, which are equal to 3.2 and 3.3.

** 40 c.c. of erythrocytes, containing 2 gm. of N_2 (equivalent to 12 gm. of protein), cause less than 0.004° rise of temperature of 250 gm. of water, or less than 1 cal., therefore, for 1 gm. of protein, less than 0.1 cal.

and also the activity of life as such do not signify work in the thermodynamical sense of the word. On the other hand, however, we observe that the interruption of metabolism inhibits all visible phenomena in the cell, movements of protoplasm, cell-divisions, secretions, etc., but that life itself can be preserved for a certain time. The plant physiologist Pfeffer has advanced the theory [120] that aërobic cells were capable of anaërobic metabolism on interruption of oxygen supply, since the first stages of the molecule breakdown occurred without the intervention of oxygen. This anoxidative cleavage was to be a sort of energetic substitute of respiration in absence of oxygen. But I found that this hypothesis proved correct only in regard to muscle, *i.e.*, the metabolism of carbohydrate in this organ. If we deprive respiring blood cells or aërobic bacteria of oxygen, no metabolism combined with perceptible heat-production can be found any longer,[76, 77] nor does there occur an accumulation of anaërobic metabolites under these conditions. This follows from the fact that in these cells respiration is not increased after a long anaërobiosis, in striking contrast with muscle. Here the metabolite, the lactic acid, accumulated on deprival of oxygen, causes afterwards a strong increase of oxygen respiration. Now although aërobic bacteria and blood cells do not show any energetic metabolism on deprival of oxygen, they are not killed immediately, but injured progressively, and this so much the more, the higher the temperature is during the absence of oxygen.

The question of the significance of energy exchange of non-working cells, *i.e.*, resting respiration, can therefore only be answered by hypothesis. For the resting respiration of muscle I found a plausible explanation, that of assisting the readiness for work. But it is an open

question how far one may adopt a similar theory for cells doing no work in the physical sense. Following up an idea of Warburg,[139] I advanced some time ago the general hypothesis [81] that in consequence of the fluid state of the protoplasm and the instability of the cell-stuffs, voluntary events of physical and chiefly of chemical nature are going on continuously which aim at a balance of the existing potentials of energy. Since life requires the continuation of these potentials of energy, work must be performed continuously for the prevention or reversion of these spontaneous changes. In a dead system it would be possible to preserve a mixture of such active bodies, apart from their physical and chemical balance, by delaying at will the reactions by resistances. In the living cell, however, this could be obtained only by continuous cycles. But we may assume besides that, just as in muscle, all the other work of the cells, such as secretion, concentration of substances, and the like, is always made possible by the same metabolism as resting respiration.

While we are here generally dependent on hypotheses, we derive more satisfaction from the close study of those cases where metabolism is utilized in a definite manner for the performance of work in the cell. Now we shall study some cases which deal with the transformation into chemical work, *i.e.*, coupled reactions.

The first case concerns the chemosynthetic assimilation of carbon dioxide in the nitrifying bacteria. As is known, in 1890 the Russian bacteriologist Winogradsky[162] discovered a number of different kinds of bacteria which thrive in a purely mineral culture fluid. By an oxidation of inorganic compounds they are also able to obtain the energy for the assimilation of carbon for the building up of their bodies. Of these kinds, the nitrifying bacteria

of the soil are known best and are also most important for the household of nature. Winogradsky discovered two kinds of these bacteria. One of these, the nitrite-forming bacteria (nitrosomonas), shows the reaction

$$NH_4^+ + 3\,O = H^+NO_2^- + H_2O + H^+ + 70{,}000 \text{ cals.}$$

The other, the nitrate-forming (nitromonas),

$$NO_2^- + O = NO_3^- + 21{,}700 \text{ cals.}$$

Now, since in these bacteria there is a complete separation of energetic metabolism from the assimilating metabolism, I investigated here more closely the laws concerning the energy exchange. It tempted me so much the more since these interesting bacteria have never been studied before from the physiological standpoint. I only want to discuss here one point, *i.e.*, the relations of these oxidations to the assimilation of carbon dioxide in the bacteria. We can find it out qualitatively by this method: We determine, for instance, the hourly consumption of oxygen of the nitrate-forming bacteria in a solution of phosphate of a hydrion concentration of $10^{-8.4}$, containing at first some carbonate, in two samples. In one of these we leave the carbon dioxide, while we make the other sample free of it by shaking it together with caustic soda in a confined space. We then remove the carbon dioxide in this way first from the air and then gradually deprive the solution of it. We thus find that in the sample containing CO_2 the respiration slowly rises in consequence of the increasing number of bacteria; in the second, however, it decreases continually. The hydrion concentration of the solution remains nearly constant due to phosphate. (Fig. 8.)

In order to measure the amount of assimilation of carbon dioxide quantitatively, the organic carbon formed within a certain interval was determined by

Messinger's technique [72a] and compared with the oxidized nitrite. These measurements were taken by Winogradsky only on the nitrite-forming bacteria. The faculty of the nitrate-forming bacteria for the assimilation of carbonic acid had not been proven by him, but only been assumed, analogous to the nitrite-forming

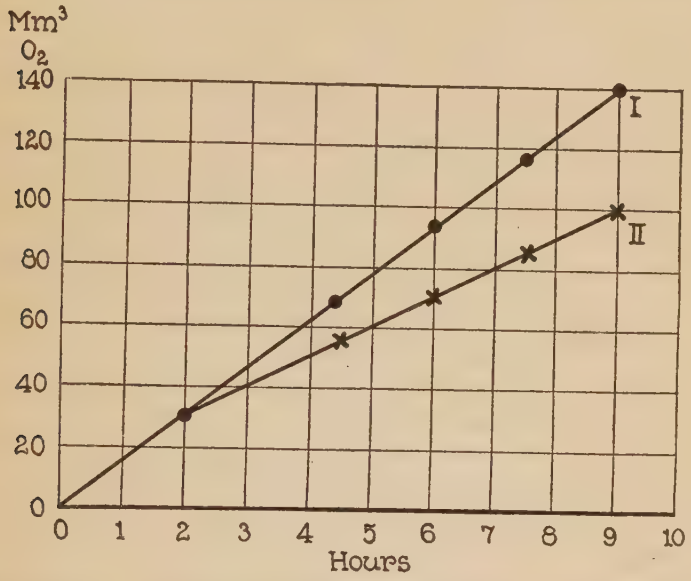


FIG. 8.—*Oxygen consumption of nitrate-forming bacteria.* I, without NaOH; II, with NaOH.

bacteria. Now with the nitrate-former it was found that the proportion $\frac{\text{mg. oxidized N}}{\text{mg. assimilated C}}$ was on the average = 135. (This means: for 135 oxidized mg. of nitrogen 1 mg. of carbon is assimilated.)

This proportion was, however, not constant. It was lower in the presence of a small concentration of nitrate than of a higher one, *i.e.*, in the beginning of cultivation more carbon dioxide was assimilated by the oxidation of the same amount of nitrate. With a low concentration of

nitrate, the quotient was quite accurate at 100. If we now assume that during the assimilation glucose is formed, this requires per atom of carbon 112,000 cals.

$$CO_2 + H_2O = 1/6\ (C_6H_{12}O_6) - 112{,}000 \text{ cals.}$$

If we take the quotient $\frac{N}{C} = 100$ there must be used 1300 cals. per mol. oxidized KNO_2 for the assimilation.* Since the molecular heat of the reaction $NO'_2 + O = NO'_3$ amounts to 21,700, the 1300 cals. correspond to 6 per cent. of this heat. By direct calorimetry I found indeed as the average of a series of experiments 20,400 cals. per mol. of formed nitrate, *i.e.*, about 5 per cent. less than corresponds to the heat of reaction. Although these heat measurements cannot be sufficiently accurate to determine from them the quantitative amount of the assimilated carbon the results found directly have been hereby confirmed. They prove at least that no other processes of energetic importance occur in this case. The result is especially remarkable if we compare it with the carbon assimilation of the nitrite-forming bacteria. Winogradsky already published some quantitative experiments on this subject. Here we find, according to his measurements, the quotient $\frac{\text{mg. N}}{\text{mg. C}} = 35$, that means only about one-third of the other (1 mg. C assimilated by oxidation of only 35 mg. N). But this does not correspond at all with a more favorable assimilative efficiency of oxidation, but rather with exactly the same, for the heat of reaction is three times as large per atom N, and therefore the yield of energy also in this case only 6 per cent.

This comparison should not really have been made between the rates of heat production, but between the free energies. They cannot be accurately determined

*$\frac{14 \times 112{,}000}{12 \times 100} = 1300$ cals.

under the conditions of the experiment. But from the computation it is found that the proportion of the free energies, just as that of the total energies, is about 3 : 1.

These experiments with nitrifying bacteria are to serve as a prelude for the discussion of the important results which Warburg and his co-worker Negelein obtained in regard to the assimilation of nitrates by green plants, in their experiments with the alga Chlorella. As is known, in nature nitrogen passes through a cycle. In this cycle the energetically most important changes comprise, in the first place, the oxidation of ammonia, derived from animal putrefaction, into saltpeter through the activity of the nitrifying bacteria of the soil, just described. Secondly, an exactly opposite change takes place through the plant cells, *i.e.,* the reduction of nitrate to ammonia and the amino group of protein. The essential factor from the standpoint of energetics is here the reduction of NO'_3 to NH_3. The linkage of ammonia to the carbon chain is biochemically of great importance, but is passing almost without any change of energy. The reduction of nitric acid to ammonia corresponds of course to the sum of the two equations above in reversed order:

$$HNO_3 + H^+ + H_2O = NH_4^+ + 2\,O_2 - 91{,}700 \text{ cals.}$$

And indeed, in the reduction of nitrate through the plant we have to do with exactly the same processes which were found in the nitrifying bacteria, only coupled in opposite order.

In order to increase the generally slight nitrate reduction in the alga, Warburg used a trick. The velocity of reduction depends on the concentration of undissociated HNO_3 molecules because they alone can penetrate into the

cell. Since the algæ cannot bear any high acid concentrations, Warburg put them into a nitrate mixture of $\frac{n}{10}$ of sodium nitrate and nitric acid, by which the dissociation of nitric acid is strongly depressed. If you now watch the nitrate reduction in the dark, the respiratory quotient, being exactly unity in the solution free from nitrate, rises considerably. Simultaneously with the formed ammonia a certain amount of carbon dioxide is evolved, not derived from the respired oxygen. First there is no quantitative relation between this carbon dioxide and ammonia, because, as is very probable, part of the ammonia is used immediately for the formation of amino-acids. But when the N-requirement of the cells is covered, a constant relation is obtained of 2 mols. CO_2 excreted and 1 mol. NH_3. Here the reaction takes place,

$$HNO_3 + H_2O + 2C = NH_3 + 2\,CO_2$$

C meaning carbon compound from the reduction stage of free carbon. We may assume this to be sugar.

There are therefore two processes coupled with each other, the endothermic reduction of nitric acid with the exothermic oxidation of carbon or a carbon compound. For the chemical work done, the total energy expressed in the reaction heat is here of less interest than the free energy. For the conditions of the experiment in acid solution we calculated the *free energy* of the nitrate reduction at minus 68,000 cals. according to the equation:

$$NO_3^- + H_2O + 2H^+ = NH_4^+ + 2\,O_2 - 68{,}000 \text{ cals.}$$

On the other hand we have the oxidation:

$$2\,O_2 + 2C = 2CO_2 + 2 \times 115{,}000 = 230{,}000 \text{ cals.}$$

By the addition of the two equations we obtain the summary:

$$NO_3^- + H_2O + 2H^+ + 2C = NH_4^+ + 2CO_2 + 162{,}000 \text{ cals. of free energy.}$$

Of the oxidation energy of carbon, 230,000 cals., only 68,000 cals. are utilized, therefore the yield of energy amounts to 30 per cent. This reaction agrees, in regard to energetics, exactly with that of gunpowder. The carbon is here also burnt to carbon dioxide at the expense of the nitrate oxygen. The oxygen causing the oxidation of the carbon is derived, as is clear, from nitric acid and water, but not from the free atmosphere. Consequently the oxidation mechanism is greatly changed. For example, the consumption of the free oxygen in the alga cell is only slightly inhibited by cyanide, while the reduction of nitrate is, however, inhibited by a $\frac{n}{1{,}000{,}000}$ KCN solution.

This process, the gunpowder reaction, so to say, is completely changed in the light. Not only much more ammonia is now formed, but beside it there also appears an excess of oxygen instead of carbon dioxide. In the light, the reduction of carbon dioxide occurs, as is known, according to the equation:

$$2CO_2 = 2C + 2\,O_2 - 230{,}000 \text{ cals. (free energy).}$$

This drops out from the balance of the reaction just mentioned and only the process remains:

$$2H^+ + NO_3^- + H_2O = NH_4^+ + 2\,O_2 - 68{,}000 \text{ cals. of free energy.}$$

Here, therefore, the supply of external energy is necessary. In the light radiation alone affords the energy for this reduction. Nevertheless, reduction proceeds by way of carbonic acid, a fact which could be decided by an interesting experiment. Through narcotics the assimila-

tion of carbonic acid is very strongly inhibited, the nitrate reduction only very slightly. If a narcotized cell is exposed to light, the same amount of gas is excreted as by unnarcotized cells. This extra gas, however, is no longer oxygen, but carbon dioxide, the very one which would have been intermediately assimilated in absence of narcotics. CO_2 here takes the part of an intermediary body through which the energy of radiation is transferred on the reduction of nitrate.

The nitrate reduction, taking place in the light, has led us to the assimilation of carbon dioxide, and draws our attention to one of Warburg's most recent works (performed in coöperation with Negelein) [149, 158] in which the exchange of energy is determined on the assimilation of carbon dioxide itself. We notice there that under suitable experimental conditions we can obtain a yield of work surpassing everything we have learned so far of chemical metabolism. Measuring, as Warburg did, with utmost precision the total radiation absorbed by the suspension of the algæ, and at the same time the amount of assimilated carbon dioxide, we find that the utilization of radiation, thus calculated, is by no means constant. In the first place the photochemical efficiency depends on the intensity of illumination. It is the greater, the less the light. The maximum efficiency can therefore only be determined as the limit under the very lowest intensity of light. Second, the algæ among themselves show a different capacity of utilization. If they have been kept in weak light for a long time, dark colored cells are obtained, containing abundant chlorophyll, which possesses an especially high power of assimilation. In this case the photochemical yield has been 60 per cent. in yellow light on the average in a long series of experiments. It is higher than any known photo-

chemical yield of energy measured so far. Warburg sees the cause for the decrease of efficiency under a high intensity of illumination in the larger amount of sugar formed hereby in the cells. The reduction of carbon dioxide into sugar is surely passing along the structural surfaces which are covered with chlorophyll molecules. We conclude this from the fact that the assimilation is extremely sensitive to the effect of narcotics, which is to be explained by a driving off of the substrate from the solid surfaces of the cell. But if as a result of a larger amount of sugar in the cell, part of the surface is covered with it, the carbon dioxide finds no place. If a ray of light strikes upon a chlorophyll molecule which does not happen to touch a molecule of carbon dioxide at that moment, the radiant energy is lost. For the existence, the "span of life," of a molecule rich in energy, which has absorbed radiation and can transform it into chemical work, amounts to only 10^{-8} seconds. This shows how dense the covering of the chlorophyll layer with carbon dioxide must be, so that the greatest part of radiation may be converted into chemical energy.

Another important result can be derived on the basis of Planck's theory of quanta, or more directly from Einstein's "law of equivalents," deduced from it.[30] As is known, the "theory of quanta" states that radiant energy can only be discontinuously absorbed or emitted in quanta in proportion to the oscillation frequency of the light. The single quantum is equal to $h\nu$, h meaning the universal Planck constant and ν the frequency of oscillation. According to Einstein's "law of equivalents," one molecule can always take in only one quantum at a time, and can only be decomposed by the light when $h\nu$ is larger

than or equal to the energy necessary for the breakdown of the molecule.

$$N_0 h\nu \geqq U$$

U means here the work required for the decomposition of 1 gm. mol., and N_0 means Avogadro's constant. U amounts to 115,000 cals. for the conversion of 1 molecule of CO_2 into $^1/_6$ molecule of glucose.

On the other hand, for yellow light there is $N_0 h\nu =$ 49,000 cals.

$$(N_0 = 6.18 \times 10^{23};\ h = 1.56 \times 10^{-24}\ \text{cals.})$$

Einstein's theorem is therefore not directly realized. U is more than twice as large as $N_0 h\nu$, and we must conclude from this that at least 3 absorbing molecules are required for the reduction of 1 molecule of carbonic acid. But the molecules absorbing light are chlorophyll, therefore at least every 3 chlorophyll molecules must have taken up one quantum of energy and be chemically changed for the assimilation of 1 molecule of carbon dioxide.

But, finally, another most important conclusion follows: If the number of quanta (n), which are required for the reduction of 1 mol. CO_2 is constant, then the efficiency of radiation ("the photochemical yield"), must evidently ascend to the red end of the spectrum in reversed proportion to ν, *i.e.*, proportional to the wavelength, because $N_0 h\nu$ grows proportionately to ν, while U remains unchanged. This consequence of Einstein's law has been exactly fulfilled in the assimilation of CO_2 according to Warburg. In contradiction to all the former statements, which had shown more or less that assimilation was taking place exclusively or predominantly in the absorption bands,* Warburg demonstrated that there is

* It is a matter of course that assimilation is only taking place where light is being absorbed (Draper's law).

no influence of definite spectral regions upon the utilization of radiation, but that the latter follows the relation of quanta, the degree of efficiency continuously decreasing from the red towards the blue end.

At 660$\mu\mu$ (red light) the maximum efficiency is 63.5 per cent. At 578$\mu\mu$ (yellow, mercury line) the maximum efficiency is 53.5 per cent. At 436$\mu\mu$ (blue, mercury line) the maximum efficiency is 34.8 per cent.

The comparison is still more accurate if the photochemical yield is measured on the same cells for different spectral lines. Thus the proportion of the yields for $\frac{660\mu\mu}{578\mu\mu}$ is found to be 1.13, while that of the wave-lengths is 1.14, agreeing perfectly. For $\frac{578\mu\mu}{478\mu\mu}$ the proportion of the yields is 1.55, that of the wave-lengths 1.32. Warburg assumes that the cause for this latter deviation lies in the fact that in the blue also the yellow pigment—xanthophyll—absorbs, but possesses weaker assimilative power. The yield of energy is therefore lowered a little more than Einstein's law would predict. If we now assume that the measured maximum efficiencies are really the highest values of the yield of energy, we can then calculate the number of quanta required for the assimilation of 1 mol. of CO_2.

For example, for red light the result is: 115,000 utilized cals. For these $\frac{115{,}000 \times 100}{63.5} = 184{,}000$ cals. must be supplied. On the other hand, for 660$\mu\mu$ ($\nu = 454 \times 10^{12}$) a quantum per mol. is 43,700 cals. The number of quanta for the reduction of 1 mol. CO_2 is then $\frac{181{,}000}{43{,}700} = 4.1$. In the same way we obtain for yellow light 3.8, for blue 4.7, as average results of numerous experiments. There

are therefore found four quanta needed for assimilation of CO_2. Therefore the highest utilization of light is reached in the case of $4N_0h\nu = U$, which equation is fulfilled at the red end of the spectrum, while in the other parts of the spectrum we have $4N_0h\nu > U$. At the same time this relation gives the exact explanation for the red limit of assimilation.

This lecture started with the difficult question, which cannot yet be completely answered, what purposes the chemical exchange of energy serves. Finally the study of some measured exchanges of energy led us to the fundamental problem of cell energetics, *i.e.,* the storage of the sun's energy in green plants, where we obtain a very valuable result. We have seen that the greatest part of the radiant energy can be changed into chemical work under proper conditions. At the same time we find a certain allusion to the chemical mechanism of the assimilation of carbon dioxide by Warburg's discovery that the simple relation of the quanta theory is here fulfilled and that probably four energy quanta are required for the conversion of one molecule of carbonic acid. This result may afford us the hope that we shall also succeed in finding a satisfactory answer concerning the utilization of oxidation energy in the chemical metabolism of the cell.

BIBLIOGRAPHY

[1] ARNOLD: Eine Farbenreaktion von Eiweisskörpern mit Nitroprussidonatrium, *Zeitschr. physiol. Chem.*, *70*, 300 (1911).

[2] ARNOLD: Über den Cysteingehalt tierischer Organe, *Zeitschr. physiol. Chem.*, *70*, 314 (1911).

[3] ATWATER: Neue Versuche über Stoff—und Kraftwechsel im menschlichen Körper, *Ergeb. Physiol.*, *3* (1), 497.

[4] BATELLI and STERN: Methoden zur Bestimmung der Atmung tierischer Gewebe, *Abderhalden's Handb. d. biochem. Arbeitsmethoden*, III, 444 (1910).

[5] BUCHNER, E., BUCHNER, H., and HAHN, M.: Die Zymase-Gärung, München und Berlin, 1903.

[6] CLAESSON: Über Thioglykolsäure, *Liebig's Ann. d. Chem.*, *187*, 120.

[7] COFFEY, S.: Linolenic and Linolic Acid, *Jour. Chem. Soc.*, *119*, 1152, 1408 (1921).

[8] DAKIN: Oxidations and Reductions in the Animal Body, Second Edition, Longmans, Green & Co., London, 1922.

[9] DAKIN, H. D.: The Oxidation of Butyric Acid by Means of Hydrogen Peroxide with Formation of Acetone, Aldehydes, and Other Products, *Journ. Biol. Chem.*, 1908, *4*, 77.

[10] DAKIN, H. D.: A Comparative Study of the Oxidation of the Ammonium Salts of Saturated Fatty Acids with Hydrogen Peroxide, *Journ. Biol. Chem.*, 1908, *4*, 227.

[11] DAKIN, H. D.: The Oxidation of Leucin, α-Amido-Isovaleric Acid, and of α-Amido-*n*-Valeric Acid with Hydrogen Peroxide, *Journ. Biol. Chem.*, 1908, *4*, 63.

[12] DAKIN, H. D.: Note of Oxidation of Glutamic and Aspartic Acids by Means of Hydrogen Peroxide, *Journ. Biol. Chem.*, 1909, *5*, 409.

[13] DAKIN, H. D., and DUDLEY, H. W.: An Enzyme Concerned with the Formation of Hydroxy Acids from Ketonic Aldehydes, *Journ. Biol. Chem.*, *14*, 321; *15*, 463; *16*, 505 (1913).

[14] DIXON and TUNNICLIFFE: Oxidation of Reduced Glutathione and Other Sulphydryl Compounds, *Proc. Roy. Soc. B.*, *94*, 266 (1923).

[15] EMBDEN, KRAUS, and OPPENHEIMER: Über Milchsäurebildung in der künstlich durchströmten Leber., *Biochem. Zeitschr.*, *45*, 1, 30 (1912).

[16] EMBDEN, BALDES, and SCHMITZ: Über den Chemismus der Milchsäurebildung aus Traubenzucker im Tierkörper, *Biochem. Zeitschr.*, *45*, 108 (1912).

[17] EMBDEN and LAQUER: Über die Chemie des Lactacidogens, *Zeitschr. physiol. Chem.*, *93*, 94 (1914), *98*, 181 (1917).

[18] EMBDEN and LAWACZEK: Über die Bildung anorganischer Phosphorsäure bei der Kontraktion des Froschmuskels, *Biochem. Zeitschr.*, *127*, 181 (1922).

[19] EMERY and BENEDICT: The Heat of Combustion of Compounds of Physiological Importance, *Am. Journ. Physiol.*, *28*, 301 (1911).

[20] EULER and KARLSSON: Zur Kenntnis der Gärungsbeschleunigungen, *Biochem. Zeitschr.*, *130*, 550 (1922).

[21] Euler and Lindberg: Gärungsaktivatoren, *Biochem. Zeitschr., 133,* 218 (1922).

[22] Fletcher, W. M.: The Survival Respiration of Muscle, *Journ. Physiol., 23,* 410 (1898–99).

[23] Fletcher, W. M.: On the Alleged Formation of Lactic Acid in Muscle during Autolysis and in Postsurvival Periods, *Ibid., 43,* 281 (1911).

[24] Fletcher, W. M.: Lactic Acid Formation, Survival Respiration, and Rigor Mortis in Mammalian Muscle, *Ibid., 47,* 361 (1913).

[25] Fletcher, W. M.: The Relation of Oxygen to the Survival Metabolism of Muscle, *Ibid., 48,* 474 (1914).

[26] Fletcher, W. M.: CO_2 Discharge of Excised Tissues, *Journ. Physiol., 23* (Suppl.), 15, 16.

[27] Fletcher, W. M., and Hopkins, F. G.: Lactic Acid in Amphibian Muscle, *Ibid., 35,* 247 (1907).

[28] Fletcher, W. M., and Brown, G. M.: The Carbon Dioxide Production of Heat Rigor in Muscle and the Theory of Intramolecular Oxygen, *Ibid., 48,* 177 (1914).

[29] Fletcher, W. M., and Hopkins, F. G.: The Respiratory Process in Muscle and the Nature of Muscular Motion, *Proc. Roy. Soc. B., 89,* 444 (1917).

[30] Gerlach, W.: Die experimenteller Grundlagen der Quantentheorie, Heft 58, Sammlung Vieweg (1921).

[31] Harden, Arthur: Alcoholic Fermentation, Second Edition, Longmans, Green & Co., London (1914).

[32] Harden, Arthur, and Young, W. J.: The Influence of Phosphates on the Fermentation of Glucose by Yeast-juice (preliminary communication, June, 1905), *Proc. Chem. Soc., 21,* 189 (1905).

[33] Harden, Arthur, and Young, W. J. (1906): The Alcoholic Ferment of Yeast-juice, Part II; The Co-ferment of Yeast-juice, *Proc. Roy. Soc. B., 78,* 369–375.

[34] Harden, Arthur, and Young, W. J. (1908): The Alcoholic Ferment of Yeast-juice, Part III; The Function of Phosphates in the Fermentation of Glucose by Yeast-juice, *Proc. Roy. Soc. B., 80,* 299–311.

[35] Harden, Arthur, and Young, W. J. (1910): The Alcoholic Ferment of Yeast-juice, Part V; The Function of Phosphates in Alcoholic Fermentation, *Proc. Roy. Soc. B., 82,* 321–330.

[36] Harden, Arthur, and Young, W. J. (1911): The Alcoholic Ferment of Yeast-juice, Part VI; The Effect of Arsenates and Arsenites on the Fermentation of the Sugars by Yeast-juice, *Proc. Roy. Soc. B., 83,* 451–475.

[37] Harden, Arthur, and Young, W. J. (1911): Über die Zusammensetzung der durch Hefepressaft gebildeten Hexosephosphorsäure, I, *Biochem. Zeitschr., 32,* 173–176.

[38] Harden, Arthur, and Maclean: On the Alleged Presence of an Alcoholic Enzyme in Animal Tissues and Organs, *Journ. Physiol., 42,* 64 (1911).

[39] Harden, Arthur, and Henley, Francis Robert: The Effect of Acetaldehyde and Methylene Blue on the Fermentation of Glucose and Fructose by Yeast-juice and Zymin in Presence of Phosphate and Arsenate, *Biochem. Journ., 15,* 175–185 (1921).

[40] Heffter: Die reduzierenden Bestandteile der Zelle, *Med. nat. Arch.* (Urban und Schwarzenberg) *1,* 81 (1908).

[41] Helmholtz: Über die Wärmeentwicklung bei der Muskelaktion, I, *Müller's Archiv.* (1847).

[42] HERMANN: Allgemeine Muskelphysik, *Handb. d. Physiol., 1*, 2 (1879).
[43] HILL, A. V.: The Position Occupied by the Production of Heat in the Chain of Processes Constituting a Muscular Contraction, *Journ. Physiol., 43*, 1 (1911).
[44] HILL, A. V.: The Heat Production of Surviving Amphibian Muscles, during Rest, Activity, and Rigor, *Journ. Physiol., 44*, 466 (1912).
[45] HILL, A. V.: The Energy Degraded in the Recovery Processes of Stimulated Muscles, *Journ. Physiol., 46*, 28 (1913).
[46] HILL, A. V.: The Oxidative Removal of Lactic Acid, *Proc. Physiol. Soc.* in *Journ. Physiol., 48, X*, (1914).
[47] HILL, A. V.: Die Beziehung zwischen der Wärmebildung und den im Muskel stattfinden chemischen Prozessen, *Ergeb. Physiol., 15*, 340 (1916).
[48] HILL, A. V.: Mechanism of Muscular Contraction, *Physiol. Reviews, 2*, 310 (1922).
[49] HILL, A. V., and HARTREE: The Four Phases of Heat Production of Muscle, *Journ. Physiol., 54*, 84 (1920).
[50] HILL, A. V., and HARTREE: The Regulation of the Supply of Energy in Muscular Contraction, *Journ. Physiol., 55*, 133 (1921).
[51] HILL, A. V., and HARTREE: The Nature of the Isometric Twitch, *Journ. Physiol., 55*, 389 (1921).
[52] HILL, A. V., and HARTREE: The Recovery Heat Production in Muscle, *Journ. Physiol., 56*, 367 (1922).
[53] HILL, A. V., and LUPTON: Muscular Exercise, Lactic Acid, and the Supply and Utilization of Energy, *Quart. Journ. Med., 16*, 135 (1923).
[54] HILL, A. V., and BROWN: The Oxygen Dissociation Curve of Blood and Its Thermodynamical Basis, *Proc. Roy. Soc. B., 94*, 292 (1923).
[55] HILL, A. V., LONG, and LUPTON: Lactic Acid in Human Muscle, *Journ. Physiol., 57, Proc.* 44 (1923).
[56] HILL, A. V., LONG, and LUPTON: The Removal of Lactic Acid during Recovery from Muscular Exercise in Man, *Journ, Physiol., 57, Proc.* 47 (1923).
[57] HILL and MEYERHOF: Über die Vorgänge bei der Muskelkontraktion, Erster Teil, der Mechanismus der Muskelkontraktion, *Ergeb. Physiol., 20* (II), 1, (1923).
[57a] HÖBER, R.: Physikalische Chemie der Zelle und Gewebe, 5. Aufl. Leipzig (1922–23).
[58] HOPKINS, F. G.: Some Oxidation Mechanisms of the Cell, *Johns Hopkins Hospital Bull., 32*, 321 (1921).
[59] HOPKINS, F. G.: On an Autoxidizable Constituent of the Cell, *Biochem. Journ., 15*, 286 (1921).
[60] HOPKINS, F. G., and DIXON, M.: On Glutathione, II; a thermostable Oxidation Reduction System, *Journ. Biol. Chem., 54*, 527 (1922).
[61] KROGH, A.: The Relative Value of Fat and Carbohydrate as Source of Muscular Energy, *Biochem. Journ., 14*, 290 (1920).
[62] LANGMUIR: *Journ. Am. Chem. Soc., 38*, 2247 (1916).
[63] LAQUER, F.: Über die Bildung von Milchsäure und Phosphorsäure im Froschmuskel, *Zeitschr. physiol. Chem., 93*, 60 (1914).
[64] LAQUER, F.: Über den Abbau der Kohlehydrate im quergestreiften Muskel, *Zeitschr. physiol. Chem., 116*, 169 (1921).
[64a] LEBEDEW: Die Darstellung des aktiven Hefesaft durch Maceration, *Zeitschr. physiol. Chem., 73*, 447 (1911).
[65] LEVENE and MEYER: On the Oxidation of Tissues on Hexoses, *Journ. Biol. Chem., 15*, 65 (1913).

[65a] Loeb, J.: Proteins and the Theory of Colloidal Behavior, New York and London, 1922.

[66] Loeb, J.: Die chemische Entwicklungserregung des tierischen Eies, Berlin, 1909, J. Springer.

[67] Löffler, W., and Spiro, K.: Über Wasserstoff und Hydroxylionen-Gleichgewicht in Lösungen, II; *Helvetica Chemica Acta, 2,* 533 (1919).

[68] Lusk, G.: The Elements of the Science of Nutrition, Philadelphia and London, Second Edition (1909).

[69] Mandel and Lusk, G.: Lactic Acid in Intermediary Metabolism, *Am. Journ. Physiol., 16,* 129 (1906).

[70] Mathews and Walker: Spontaneous Oxidation of Cysteine, *Journ. Biol. Chem., 6,* 21 (1909).

[71] Mathews and Walker: The Action of Cyanides and Nitriles on the Spontaneous Oxidation of Cysteine, *Journ. Biol. Chem., 6,* 29 (1909).

[72] Mayer, R.: Die Mechanik der Wärme, Ostwald's Klassiker der exakten Wissenschaften, Nr. 180.

[72a] Messinger, Berl: *Chem. Ber., 21,* 1910, *23,* 1757.

[73] Meyer, H. H.: Zur Theorie der Alkohol Narkose, *Arch. Exper. Path. und Pharm., 42,* 109 (1899).

[74] Meyerhof, O.: Die Atmung der Seeigeleier (*Strongylocentrotus lividus*) in reinen Chlornatriumlösungen, *Biochem. Zeitschr., 33,* 291 (1911).

[75] Meyerhof, O.: Untersuchungen über die Wärmetönung der vitalen Oxydationsvorgänge in Eiern, I–III, *Biochem. Zeitschr., 35,* 246 (1911).

[76] Meyerhof, O.: Über Wärmetönung chemischer Prozesse in lebenden Zellen, (Versuche an Blutzellen), *Pflüger's Arch. ges. Physiol., 146,* 159 (1912).

[77] Meyerhof, O.: Über den Energiewechsel von Bakterien, *Sitzungsberichte d. Heidelberger Akademie d. Wissenschaften, 3,* (1912).

[78] Meyerhof, O.: Über scheinbare Atmung abgetöteter Zellen durch Farbstoffreduktion, (Versuche an Acetonhefe), *Pflüger's Arch. ges. Physiol., 149,* 250 (1912).

[79] Meyerhof, O.: Über Hemmung von Fermentreaktionen durch indifferente Narkotika, *Pflüger's Arch. ges. Physiol., 157,* 251 (1914).

[80] Meyerhof, O.: Über Hemmung der Wasserstoffsuperoxydzersetzung des kolloidalen Platins durch indifferente Narkotika, *Pflüger's Arch. ges. Physiol., 157,* 307 (1914).

[81] Meyerhof, O.: Zur Energetik der Zellvorgänge, Vandenhoek und Ruprecht, Göttingen, 1 (1913).

[82] Meyerhof, O.: Untersuchungen über den Atmungsvorgang nitrifizierender Bakterien, I, Die Atmung des Nitratbildners, *Pflüger's Arch. ges. Physiol., 164,* 353 (1916).

[83] Meyerhof, O.: Untersuchungen über den Atmungsvorgang nitrifizierender Bakterien, II, Beeinflussung der Atmung des Nitratbildners durch chemische Substanzen, *Pflüger's Arch. ges. Physiol., 165,* 229 (1916).

[84] Meyerhof, O.: Untersuchungen über den Atmungsvorgang nitrifizierender Bakterien, III, Die Atmung des Nitritbildners und ihre Beeinflussung durch chemische Substanzen, *Pflüger's Arch. ges. Physiol., 166,* 240 (1917).

[85] MEYERHOF, O.: Untersuchungen zur Atmung getöteter Zellen. I. Mitteilung. Die Wirkung des Methylenblaus auf die Atmung lebender und getöteter Staphylococcen nebst Bemerkungen über den Einfluss des Milieus, der Blausäure und Narkotika, *Pflüger's Arch. ges. Physiol., 169*, 87 (1917).

[86] MEYERHOF, O.: Untersuchungen zur Atmung getöteter Zellen. II. Mitteilung. Der Oxydationsvorgang in getöteter Hefe und Hefeextrakt, *Pflüger's Arch. ges. Physiol., 170*, 367 (1918).

[87] MEYERHOF, O.: Untersuchungen zur Atmung getöteter Zellen. III. Mitteilung. Die Atmungserregung in gewaschener Acetonhefe und dem Ultrafiltrationsrückstand von Hefemazerationssaft, *Pflüger's Arch. ges. Physiol., 170*, 428 (1918).

[88] MEYERHOF, O.: Neuere Untersuchungen über die Beziehungen zwischen Atmung und Gärung, *Medizinische Klinik, 18*, 1 (1918).

[89] MEYERHOF, O.: Über das Vorkommen des Coferments der alkaholischen Hefegärung im Muskelgewebe und seine mutmassliche Bedeutung im Atmungsmechanismus, *Zeitschr. physiol. Chem., 101*, 165 (1918).

[90] MEYERHOF, O.: Über das Gärungscoferment im Tierkörper, *Zeitschr. physiol. Chem., 102*, 1 (1918).

[91] MEYERHOF, O.: Zur Kinetik der zellfreien Gärung, *Zeitschr. physiol. Chem., 102*, 185 (1918).

[92] MEYERHOF, O.: Notiz über Eiweissfällungen durch Narkotika, *Biochem. Zeitschr., 86*, 325 (1918).

[93] MEYERHOF, O.: Über die Atmung der Froschmuskulatur, *Pflüger's Arch. ges. Physiol., 175*, 20 (1919).

[94] MEYERHOF, O.: Über den Zusammenhang von Atmung und Gärung, *Naturwissenschaften, 16*, 253 (1919).

[95] MEYERHOF, O.: Zur Verbrennung der Milchsäure in der Erholungsperiode des Muskels, *Pflüger's Arch. ges. Physiol., 175*, 88 (1919).

[96] MEYERHOF, O.: Die Energieumwandlungen im Muskel. I. Über die Beziehung der Milchsäure zur Wärmebildung und Arbeitsleistung des Muskels in der Anaerobiose, *Pflüger's Arch. ges. Physiol., 182*, 232 (1920).

[97] MEYERHOF, O.: Die Energieumwandlungen im Muskel. II. Das Schicksal der Milchsäure in der Erholungsperiode des Muskels, *Pflüger's Arch. ges. Physiol., 182*, 284 (1920).

[98] MEYERHOF, O.: Die Energieumwandlungen im Muskel. III. Kohlehydrat und Milchsäureumsatz im Froschmuskel, *Pflüger's Arch. ges. Physiol., 185*, 11 (1920).

[99] MEYERHOF, O.: Die Energieumwandlungen im Muskel. IV. Über die Milchsäurebildung in der zerschnittenen Muskulatur, *Pflüger's Arch. ges. Physiol., 188*, 114 (1921).

[100] MEYERHOF, O.: Die Energieumwandlungen im Muskel. V. Milchsäurebildung und mechanische Arbeit, *Pflüger's Arch. ges. Physiol., 191*, 128 (1921).

[101] MEYERHOF, O.: Die Energieumwandlungen im Muskel. VI. Über den Ursprung der Kontraktionswärme, *Pflüger's Arch. ges. Physiol., 195*, 22 (1922).

[102] MEYERHOF, O.: Die Verbrennungswärme der Milchsäure, *Biochem. Zeitschr., 129*, 594 (1922).

[103] MEYERHOF, O.: Die Energieumwandlungen im arbeitenden Muskel, *Medizinische Klinik, 17*, 1 (1920).

[104] MEYERHOF, O.: Über die Rolle der Milchsäure in der Energetik des Muskel, *Naturwissenschaften, 35,* 691 (1920).

[105] MEYERHOF, O.: Neue Versuche zur Thermodynamik der Muskelkontraktion, *Naturwissenschaften, 12,* 193 (1921).

[106] MEYERHOF, O.: Über ein neues autoxydablen System der Zelle (die Rolle der Sulfhydrilgruppe als Sauerstoffüberträger), *Pflüger's Arch. ges. Physiol., 199,* 531 (1923).

[107] MEYERHOF, O.: Über Blausäurehemmung in autoxydablen Sulfhydrilsystemen, *Pflüger's Arch. ges Physiol., 200,* 1 (1923).

[107a] MEYERHOF, O.: Not yet published.

[108] MEYERHOF, O., and WARBURG, O.: Über Atmung in abgetöteten Zellen und in Zellfragmenten, Abschnitt II, Versuche an Seeigeleiern, *Pflüger's Arch. ges. Physiol., 148,* 295 (1912).

[109] MEYERHOF, O., and WEBER, H.: Beiträge zu den Oxydationsvorgängen am Kohlemodell, *Biochem. Zeitschr., 135,* 558 (1923).

[110] MEYERHOF, O., and HILL, A. V.: Über die Vorgänge bei der Muskelkontraktion, Abschnitt II, Die mechanischen und energetischen Verhältnisse bei der Muskeltätigkeit, *Ergeb. Physiol., 20,* II, 30 (1923).

[111] NEUBERG, CARL: Über den Zusammenhang der Gärungserscheinungen in der Natur. Festschrift der Kaiser Wilhelm Gesellschaft zur Förderung der Wissenschaften, J. Springer, Berlin, 1921.

[112] NEUBERG, CARL, and KARCZAG, L.: Über Zuckerfreie Hefegärungen, IV, Carboxylase ein neues Enzym der Hefe, *Biochem. Zeitschr., 36,* 68–75 (1911).

[113] NEUBERG, CARL, and OERTEL, W.: Studien über Methylglyoxalbildung, *Biochem. Zeitschr., 51,* 128–142 (1913).

[114] NEUBERG, CARL, and SCHWENK: Cofermentartige Wirkung von Salzen der α-Ketosäuren, *Biochem. Zeitschr., 71,* 135 (1915).

[115] NEUBERG, CARL, and REINFURTH, E.: Die Festlegung der Aldehydstufe bei der alkoholischen Gärung, *Biochem. Zeitschr., 89,* 365 (1918).

[115a] NEUBERG, CARL, and HIRSCH: Acetaldehyd im intermediären Stoffwechsel über lebender Muskulatur, *Biochem. Zeitschr., 134,* 415 (1922).

[116] OVERTON: Studien über Narkose, Jena (1901).

[117] PARNAS: Über das Wesen der Muskelerholung, *Zentr. f. Physiol., 30,* 1 (1915).

[118] PASTEUR: Études sur la bière, Paris, 1876.

[119] PETERS: The Heat Production of Fatigue and Its Relation to the Production of Lactic Acid in Amphibian Muscle, *Journ. Physiol., 47,* 243 (1913).

[120] PFEFFER: Pflanzenphysiologie, *1* und *2,* W. Englemann, Leipzig, 1897.

[121] ROTH and GINSBERG: Dissertation, Braunschweig (1923).

[122] SHEARER: On the Oxidation Processes of the Echinoderm Egg during Fertilization, *Proc. Roy. Soc. B., 93,* 213 (1922).

[123] SHEARER: On the Heat Production and Oxidation Processes of the Echinoderm Egg during Fertilization and Early Development, *Proc. Roy. Soc. B., 93,* 410 (1922).

[124] SLATER: The Heat of Combustion of Glycogen, with Special Reference to Muscular Contraction, International Congresss of Physiology, Edinburgh, 1923, and *Journ. Physiol., 57, Proc. B., 38* (1923).

[124a] STOHMANN: *Journ. f. praktische Chem.* (2), *50,* 385 (1894).

[125] THUNBERG, TH.: Untersuchungen autoxydabler Substanzen und autoxydabler Systeme von physiologischem Interesse, I, *Skand. Arch. Physiol., 24*, 90 (1910).

[126] THUNBERG, TH.: Untersuchungen autoxydabler Substanzen und autoxydabler Systeme von physiologischem Interesse, II, *Skand. Arch. Physiol., 24*, 94 (1910).

[127] THUNBERG, TH.: Die biologische Bedeutung der Sulfhydrilgruppen, *Ergeb. Physiol., 11*, 328 (1911).

[128] THUNBERG, TH.: Zur Kenntnis einiger autoxydabler Thioverbindungen, *Skand. Arch. Physiol., 30*, 285 (1913).

[129] TRAUBE, I.: Theorie der Osmose und Narkose, *Pflüger's Arch. ges. Physiol., 105*, 541 (1904).

[130] TREADWELL: Über die Nichtfällbarkeit des Kupfers durch Schwefelwasserstoff in zyankalihaltiger Lösung, *Zeitschr. anorg. Chem., 38*, 92 (1904).

[131] VERZAR: Der Gaswechsel des Muskels, *Ergeb. Physiol., 15*, 1 (1916).

[132] WARBURG, O.: Beobachtungen über die Oxydationsprozesse im Seeigelei, *Zeitschr. physiol. Chem., 57*, 1 (1908).

[133] WARBURG, O.: Zur Biologie der roten Blutzellen, *Zeitschr. physiol. Chem., 59*, 112 (1909).

[134] WARBURG, O.: Über die Oxydation in lebenden Zellen nach Versuchen im Seeigelei, *Zeitschr. physiol. Chem., 66*, 305 (1910).

[135] WARBURG, O.: Über Beeinflussung der Oxydationen in lebenden Zellen nach Versuchen an roten Blutkörperchen, *Zeitschr. physiol. Chem., 69*, 452 (1910).

[136] WARBURG, O.: Über Beeinflussung der Sauerstoffatmung, *Zeitschr. physiol. Chem., 70*, 413 (1911).

[137] WARBURG, O.: Über Beziehung zwischen Zellstruktur und biochemischen Reaktionen, *Pflüger's Arch. ges. Physiol., 145*, 277 (1912).

[138] WARBURG, O.: Über Hemmung der Blausäurewirkung in lebenden Zellen, *Zeitschr. physiol. Chem., 76*, 331 (1912).

[139] WARBURG, O.: Beiträge zur Physiologie der Zelle, insbesondere über die Oxydationsgeschwindigkeit in Zellen, *Ergeb. Physiol., 14*, 253 (1914).

[140] WARBURG, O.: Über die Rolle des Eisens in der Atmung des Seeigeleis, *Zeitschr. physiol. Chem., 92*, 231 (1914).

[141] WARBURG, O.: Zellstruktur und Oxydationsgeschwindigkeit nach Versuchen am Seeigelei, *Pflüger's Arch. ges. Physiol., 158*, 190 (1914).

[142] WARBURG, O.: Über Verbrennung der Oxalsäure an Blutkohle und die Hemmung dieser Reaktion durch indifferente Narkotika, *Pflüger's Arch. ges. Physiol., 155*, 547 (1914).

[143] WARBURG, O.: Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen, I, *Biochem. Zeitschr., 100*, 230 (1919).

[144] WARBURG, O.: Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen, II, *Biochem. Zeitschr., 103*, 188 (1920).

[145] WARBURG, O.: Physikalische Chemie der Zellatmung, *Biochem. Zeitschr., 119*, 134 (1921).

[146] WARBURG, O.: Über Oberflächenreaktionen in lebenden Zellen, *Zeitschr., Elektrochemie*, 70 (1922).

[147] Warburg, O.: Über die antikatalytische Wirkung der Blausäure, *Biochem. Zeitschr., 136,* 266 (1923).

[148] Warburg, O.: Über die sogenannte Autoxydation des Cysteins, *Pflüger's Arch. ges. Physiol., 200, 203,* (1923).

[149] Warburg, O.: Energieumsatz und Wellenlänge bei der Kohlensäureassimilation. *Zeitschr. physik. Chem., 106,* 191 (1923).

[150] Warburg, O., and Meyerhof: Über Atmung in abgetöten Zellen und Zellfragmenten, *Pflüger's Arch. ges. Physiol. 148,* 295 (1912).

[151] Warburg, O., and Dorner: Über Beeinflussung der alkoholischen Gärung in der Zelle und Zellpresssaft, *Zeitschr. physiol. Chem., 81,* 99 (1912).

[152] Warburg, O., and Usui: Über die Bindung von Thymol an roten Blutzellen, *Zeitschr. physiol. Chem., 81,* 175 (1912).

[153] Warburg, O., and Wiesel: Über die Wirkung von Substanzen homologer Reihen auf Lebensvorgänge, *Pflüger's Arch. ges. Physiol., 144,* 465, (1912).

[154] Warburg, O., and Meyerhof: Über Oxydation von Lecithin in Gegenwart von Eisensalz, *Zeitschr. physiol. Chem., 85,* 412 (1913).

[155] Warburg, O., and Dorner: Über Verteilungsgleichgewichte einiger indifferenter Narkotika, *Sitzungsberichte der Heidelberger Akademie der Wissenschaften, Math. nat. Klasse, Abt., B.* (1914).

[156] Warburg, O., and Negelein: Über die Reduktion der Salpetersäure in grünen Zellen, *Biochem. Zeitschr., 110,* 66 (1920).

[157] Warburg, O., and Negelein: Über Oxydation des Cysteins und andere Aminosäuren an Blutkohle, *Biochem. Zeitschr., 113,* 257 (1921).

[158] Warburg, O., and Negelein: Über den Energieumsatz bei der Kohlensäureassimilation, *Zeitschr. physik. Chem., 102,* 236 (1922).

[158a] Warburg, O., and Brefeld: Über Blutkohle und Häminkohle, *Naturwissenschaften, 11,* Heft 42, 862 (1923).

[159] Weizsacker: Arbeit und Gaswechsel am Froschherzen, *Pflüger's Arch. ges. Physiol., 141,* 452 (1911).

[160] Weizsäcker: II, Wirkung des Cyanids, *Pflüger's Arch. ges. Physiol., 147,* 135 (1912).

[161] Wieland: Über den Mechanismus der Oxydationsvorgänge, *Ergeb. Physiol., 20,* 477 (1922).

[162] Winogradsky: Die Nitrifikation, *Handb. der technischen Mykologie von Lafare, 3,* 132 (1907).

[163] Winterstein, H.: Narkose, Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere, *2,* J. Springer, Berlin, 1919.

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